

Stockholm 1044. Kungl. Boktr. P. A. Norstedt & Söner 41942

# PHARMACOLOGICAL REVIEWS

VOLUME 1

1949

Stockholm 1044 Kungl. Boktr. P. A. Norstedt & Söner 441942

# PHARMACOLOGICAL REVIEWS

## *Editorial Board*

LOUIS S GOODMAN, *Editor*

JULIUS H COMROE

A McGEHEE HARVEY

CARL A. DRAGSTEDT

OTTO KRAYER

HARRY EAGLE

CARL PFEIFFER

ALFRED GILMAN

MAURICE L TAINTER

VOLUME 1

1949

BALTIMORE, MARYLAND

---

*Reprinted with the permission of the original publishers,  
THE WILLIAMS & WILKINS COMPANY*

JOHNSON REPRINT CORPORATION  
New York, New York





# CONTENTS

## NUMBER 1, APRIL, 1949

I Metabolism of Adrenaline	Z M Bacq	1
II Pharmacology of Adrenergic Blockade	Mark Nickerson	27
III Interactions of Drugs and Plasma Proteins	Avram Goldstein	102
IV Anticholinesterase Drugs	Alfred Gilman and G B Koelle	166

## NUMBER 2, AUGUST, 1949

V The Metabolism of Barbiturates	by E W Maynert and H B van Dyke	217
VI Spinal Cord Depressant Drugs	by F M Berger, M.D	243
VII The Pharmacological Activity of Epinephrine and Related Dihydroxyphenyl-alkylamines	by A M Lands	279

## NUMBER 3, DECEMBER, 1949

VIII The Energy Metabolism of the Failing Heart and the Metabolic Action of the Cardiac Glycosides	Albert Wollenberger	311
IX Certain Aspects of the Pharmacology of the Salicylates	Paul K Smith	353
X Monofluoroacetic Acid and Related Compounds	Maynard B Chenweth	383
XI The Effects of Drugs upon the Electrical Activity of the Brain	James E P Toman and Jean P Davis	425



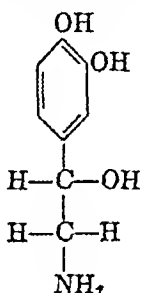
# THE METABOLISM OF ADRENALINE

Z M BACQ

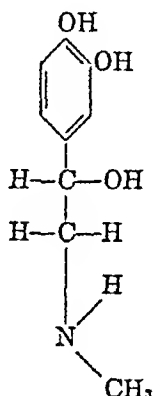
*Department of General Pathology and Therapeutics, The University of Liège, Belgium*

The purpose of this review is to bring together the useful information recently published on the normal metabolism of adrenaline in the body (see also other reviews, 1-4b)

It should be called to mind that adrenaline is physiologically liberated in the circulation in small amounts (a few  $\mu\text{g}$  per kg), that it does not pass through the liver before reaching the lungs where very little, if any, is inactivated (5), that it is distributed to all the tissues by the arterial blood, and that most of its effects do not last longer than a few minutes<sup>1</sup>



Noradrenaline



Adrenaline

The structure of the adrenaline molecule shows that there are four points of attack for enzymes or oxidizing agents: 1) the methyl group attached on the nitrogen, 2) the hydroxyl of the secondary alcohol, 3) the aminated two-carbon chain itself, and 4) the two phenolic hydroxyls

<sup>1</sup> The presence in the tissues and in the blood of noradrenaline (arterenol-aminoethanol-catechol) is becoming increasingly probable (6, 7, 8, 9, 10, 11, 12) and the separation of its two optical isomers (13) is a useful step in the elucidation of its physiological importance (14)

It seems that the properties of sympathin E (excitatory) postulated by Cannon and Rosenblueth (15) are those of noradrenaline and that sympathin I (inhibitory) is adrenaline. Thus the idea of a combination of adrenaline with specific, hypothetical cellular substances E and I is abandoned. One comes back to the first concept of Cannon and Bacq (16), sympathin is the substance, or mixture of substances, liberated by adrenergic nerve action, and not adrenaline modified by contact with cellular elements.

One should speak, as suggested by von Euler (9), of sympathin N (noradrenaline) and sympathin A (adrenaline) to describe the two sympathicomimetic substances synthesized by the tissues other than the adrenal medulla, the parotid gland of tropical toads and some modified nerve cells of Annelids, which are known to synthesize pure *l*-adrenaline (17). The metabolism of arterenol has many points in common with that of adrenaline, but it is not necessarily the same.



injections (38) There is also some evidence that adrenaline in the arterial blood may, in certain conditions, be stored by the adrenal medulla itself (39) Unfortunately, the chemical method of Shaw (30), even when modified (31), is not suitable for differentiating adrenaline from noradrenaline at the normal concentrations of these substances in tissue extracts (40, 41) With this method, only heart extracts appear to give clear indication of the presence of adrenaline as such Biological titration is certainly much more sensitive A sharp differentiation between adrenaline and noradrenaline can be obtained by simultaneously recording in the cat the responses of the sensitized nictitating membrane and of the non-pregnant uterus (10), but highly purified extracts are required and a variable proportion of the active material is lost during the process of purification Thus, when one is confronted with the problem of the titration of small amounts of adrenaline in tissue extracts, the chemical method of Shaw is found *qualitatively* unsuitable, and the physiological method *quantitatively* unreliable A more recent attempt to solve this problem is interesting By the combined use of a polyphenoloxidase from *Atropa belladonna* and the filter paper adsorption technic (so useful for amino acids), James has found it possible to differentiate adrenaline in the presence of arterenol (42) The fluorescence method is difficult and, so far, has given contradictory results (43-46)

Despite these difficulties, there is little doubt that the store of adrenaline-like substances is increased by adrenaline injections, that this phenomenon is limited, and that it may have significance in certain pathological conditions (47) It would be desirable, however, to have more quantitative data on how much adrenaline, circulating in physiological concentrations, can leave the blood and be stored in tissues in a loose, inactive combination It has been shown recently that certain tissues can store large amounts of histamine (118)

Unexpected confirmation of this storage of active adrenaline by the tissues has come from recent observations on the elimination of free adrenaline in the urine when anesthetized dogs are injected intravenously with large amounts of this amine During the injection, when renal vasoconstriction is maximal, there is little or no secretion of urine, and such urine as is secreted does not contain adrenaline The compound appears in the urine after the injection is completed, when the cardiovascular effects have disappeared, it may be present in the urine an hour after the end of the intravenous injection The peak of the excretion is reached in 30 to 40 minutes

It is a well-known fact that injected adrenaline disappears rapidly from the blood (48, 121), indeed, in certain conditions, it may disappear from the blood at a time when some physiological effects are still visible (49) The fact that free adrenaline appears in the urine for a long time after the injection appears to imply that some of the free adrenaline stored by the tissues or the blood cells is slowly released and eliminated, provided the kidney is unable to hydrolyze sulfoconjugates (see page 8 to 12)

III THE METHYL GROUP ON THE NITROGEN The source of the  $-\text{CH}_3$  attached to the nitrogen is not known, but it is not improbable that it comes from methionine, the methyl donor "par excellence" (3, 50), the use of modern tech-

A primary question is whether adrenaline is excreted unchanged or taken up the tissues and retained in the cells in some type of loose biologically combination, as is known for acetylcholine or histamine

**I EXCRETION OF UNMODIFIED ADRENALINE** It has not been reported <sup>21</sup> adrenaline when given in physiological amounts passes the kidney barrier. None of the many hypertensive substances found in the non-hydrolyzed urine of normal men or hypertensive patients exhibits properties corresponding to those of adrenaline or any catechol derivative. Surprisingly, rather high concentrations (up to  $5 \times 10^{-6}$ ) have been found in the urine of anesthetized dogs given large amounts of *dl*-adrenaline (1 to 10 mg) by intravenous injection subsequent to the administration of an adrenolytic (22) substance (933 F, yohimbine), but the quantities recovered were always small (23). <sup>2</sup> Naturally, because the quantity of adrenaline injected is enormous, the animal is often prostrate and cannot consider its condition as physiological, but the presence of adrenaline was also observed in some samples of normal urine, in several instances, when the dog was particularly excited or the blood pressure was low before injection

**II POSSIBILITY OF TISSUE STORAGE OF ACTIVE ADRENALINE** Phenolic substances rapidly leave the circulating fluids and accumulate in the cells, and it seems that adrenaline follows this rule (24, 121). A considerable proportion of adrenaline added *in vitro* to oxalated or defibrinated cat's blood enters the red cells, until equilibrium is reached. It stays active in the red cell for at least ten hours at 38° C, it can be liberated simply by laking the blood (25). There is nothing in the plasma of mammals to destroy adrenaline, on the contrary, the blood contains many substances (proteins, amino acids, ascorbic acid, glutathione, etc.) which inhibit its autoxidation. Adrenaline and all the catechol derivatives are more stable in body fluids or even in diluted plasma than in water or in sodium chloride solution of the same pH.

There is a good deal of evidence in favor of an increase in the adrenaline (or sympathin) extractable from the tissues after injection of adrenaline. It is known that adrenaline-like substances can be extracted from sympathetic nerves, the heart and various other tissues, chemical evidence corroborates physiological assays (7, 28-32). Sympathetic denervation decreases this tissue store of adrenaline-like substances (7, 32-34), but it is not yet clear whether this decrease occurs in the effector cells or is merely the result of degeneration of the postganglionic adrenergic fibers in the extracted tissues.

There is good agreement between these observations and those showing that the acetylcholine stores of various tissues innervated by cholinergic nerves disappear after section of these nerves (35-37). The concentration of acetylcholine or adrenaline-like substances in smooth muscle, heart, glands and ganglion cells appears to be a chemical constant controlled by the nervous system.

This epinephrine-like material, or absorbable chromogens (A C), as it is called by certain authors for technical reasons (31, 32, 34), increases after adrenaline

<sup>2</sup> The same phenomenon has been observed in cats injected with adrenaline (D. Richter and F. C. Mac Intosh, personal communication).

possess an aminated two carbon chain  $\left( \begin{array}{c} \text{≡}\overset{\beta}{\text{C}}-\overset{\alpha}{\text{CH}_2}-\text{N} \end{array} \right)$  If the  $\alpha$ -carbon is substituted (for example, by a methyl radical,  $\text{≡}\text{C}-\underset{\text{N}}{\underset{|}{\text{CH}}}-\text{CH}_3$ ), the amine is

not oxidized and becomes an inhibitor of the enzyme (59,60) Blaschko, Richter and Schlossman (54) re-described under the name of "adrenaline-oxidase" the tyramine-oxidase or the aliphatic amine-oxidase of Hare (61) and of Pugh and Quastel (62), the identity of these enzymatic systems is unquestionable (59) Accordingly this enzymatic system was correctly named "amine-oxidase" or monoamine-oxidase, its activity allowed satisfactory interpretation of older experiments on the metabolism of tyramine (63) and of the amines resulting from putrefaction (64), which have been confirmed in man (65)

Indirect arguments in favor of a physiological action of amine-oxidase were found in the fact that ephedrine and amphetamine, which are not oxidized by this enzyme, are excreted unchanged by the kidneys (65) and that ephedrine, presumably by its inhibitory effect on amine-oxidase, increases the amount of sympathin (in this case, very probably adrenaline) liberated by sympathetic stimulation of the perfused rabbit's ear (66)

Nevertheless, it seems that the presence of this enzyme does not provide an adequate explanation for the rapid disappearance of low concentrations of adrenaline in the circulating blood The main arguments are the following

1) Amine-oxidase is concentrated in the liver, the intestine and central nervous system In these strategic positions, it protects the organism and the nerve centers against the toxic action of the amines originating from food digestion and bacterial action (67)

2) Amine-oxidase is absent (67), or is present only in very small concentration (68), in tissues which, like the rabbit's ear, inactivate adrenaline perfused through them

3) Liver amine-oxidase requires 12 minutes to inactivate *in vitro* 50% of  $10^{-7}$  M adrenaline (69) It can be calculated that the concentration of adrenaline which saturates 50% of the enzyme is greater than  $1.5 \times 10^{-2}$  M, or 40 times that of tyramine Even if one assumes the enormous adrenaline concentration of  $10^{-4}$  M, the enzyme would act at only 1% of its optimal activity, the physiological concentration of adrenaline in the blood is about  $10^{-9}$  M Thus amine-oxidase is more prone to inactivate tyramine or aliphatic amines than to oxidize adrenaline (69) Philpot (77) correctly points out that conditions of enzyme activity *in vivo* are quite different from those *in vitro*

4) Evisceration, or temporary arrest of the circulation in the liver and the intestine where amine-oxidase is concentrated, does not increase either the intensity or the duration of action of physiological amounts of adrenaline injected intravenously in the dog (70) or in the cat (71) There is a slow process of sensitization to adrenaline after evisceration, but this phenomenon is simply

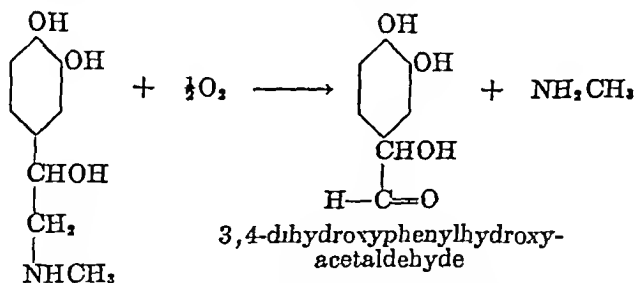


nics employing methyl groups labelled by deuterium or radioactive carbon settle this point. Indeed, it has already been observed in du Vigneaud's laboratory that radioactive carbon concentrates in the adrenals of a rat fed with methionine containing  $C^{14}$  in its methyl radical (51). On the other hand, there is no evidence that adrenaline is demethylated in the body, noradrenaline to be the precursor of adrenaline and not the first step in its inactivation should be important, however, to eliminate every uncertainty since 7-dimethylxanthines and other methylated substances are demethylated in the body (52) and since monomethylaminoethanol, the side-chain of adrenaline an excellent precursor of choline, as was shown by the aid of monomethylamine ethanol labeled with deuterium in the methyl group (53). The possibility cannot be excluded that adrenalin, or some of its oxidation derivatives in the series of adrenochrome, might be a methyl donor.

The presence of a single methyl group on the nitrogen is a basic factor in the balance between excitatory and inhibitory actions of the catecholamines (see page 21).

**IV THE SECONDARY ALCOHOL GROUP** This group is less important at first sight, but its rôle is not negligible in the oxidation to adrenochrome and beyond adrenochrome (see page 20), and in the determination of the relative potencies of the inhibitory and excitatory actions of the amines derived from catechol.

**V DEAMINATION OF THE SIDE-CHAIN** An enzyme called monoamine-oxidase, which is concentrated in the liver, the intestine and the central nervous system, inactivates adrenaline *in vitro*. Schematically, the reaction is as follows (54, 55)



This reaction is accompanied by complete physiological inactivation, methylamine, like all the aliphatic amines, has only a negligible action. The aldehyde (dihydroxyphenylacetaldehyde) resulting from amine-oxidase action on oxytyramine has vasodilator properties (56, 57), but the amounts of aldehyde eventually liberated from adrenaline by amine-oxidase in the body are too small to be of physiological interest (58). Furthermore these aldehydes are rapidly oxidized *in vivo*.

Amine-oxidase is not inactivated by cyanide, glutathione or sulfhydryl substances (54). Adrenaline is not a specific substrate for this enzyme which oxidizes (and deaminates) all amines, whether aromatic or aliphatic, which

possess an aminated two carbon chain  $\left( \begin{array}{c} \text{≡}\overset{\beta}{\text{C}}-\overset{\alpha}{\text{CH}_2}-\text{N} \end{array} \right)$  If the  $\alpha$ -carbon is substituted (for example, by a methyl radical,  $\text{≡C}-\underset{\text{N}}{\underset{|}{\text{CH}}}-\text{CH}_3$ ), the amine is

not oxidized and becomes an inhibitor of the enzyme (59,60) Blaschko, Richter and Schlossman (54) re-described under the name of "adrenaline-oxidase" the tyramine-oxidase or the aliphatic amine-oxidase of Hare (61) and of Pugh and Quastel (62), the identity of these enzymatic systems is unquestionable (59). Accordingly this enzymatic system was correctly named "amine-oxidase" or monoamine-oxidase, its activity allowed satisfactory interpretation of older experiments on the metabolism of tyramine (63) and of the amines resulting from putrefaction (64), which have been confirmed in man (65)

Indirect arguments in favor of a physiological action of amine-oxidase were found in the fact that ephedrine and amphetamine, which are not oxidized by this enzyme, are excreted unchanged by the kidneys (65) and that ephedrine, presumably by its inhibitory effect on amine-oxidase, increases the amount of sympathin (in this case, very probably adrenaline) liberated by sympathetic stimulation of the perfused rabbit's ear (66)

Nevertheless, it seems that the presence of this enzyme does not provide an adequate explanation for the rapid disappearance of low concentrations of adrenaline in the circulating blood The main arguments are the following

1) Amine-oxidase is concentrated in the liver, the intestine and central nervous system In these strategic positions, it protects the organism and the nerve centers against the toxic action of the amines originating from food digestion and bacterial action (67)

2) Amine-oxidase is absent (67), or is present only in very small concentration (68), in tissues which, like the rabbit's ear, inactivate adrenaline perfused through them

3) Liver amine-oxidase requires 12 minutes to inactivate *in vitro* 50% of  $10^{-7}$  M adrenaline (69) It can be calculated that the concentration of adrenaline which saturates 50% of the enzyme is greater than  $1.5 \times 10^{-2}$  M, or 40 times that of tyramine Even if one assumes the enormous adrenaline concentration of  $10^{-4}$  M, the enzyme would act at only 1% of its optimal activity, the physiological concentration of adrenaline in the blood is about  $10^{-9}$  M Thus amine-oxidase is more prone to inactivate tyramine or aliphatic amines than to oxidize adrenaline (69) Philpot (77) correctly points out that conditions of enzyme activity *in vivo* are quite different from those *in vitro*

4) Evisceration, or temporary arrest of the circulation in the liver and the intestine where amine-oxidase is concentrated, does not increase either the intensity or the duration of action of physiological amounts of adrenaline injected intravenously in the dog (70) or in the cat (71) There is a slow process of sensitization to adrenaline after evisceration, but this phenomenon is simply

due to a drop in body temperature, it does not occur if the eviscerated animal is kept warm (73) .

5) It is unfair to compare the effects of adrenaline injected in the portal vein with the actions of an equal dose given intravenously. In order to obtain, by the method of adrenaline injection, a true idea of the inactivating power of the tissues *in situ*, one must compare, on a distant test object, the results of intra-portal injection with injection of the same physiological amount in an artery (the femoral, for example) irrigating a mass of tissue approximately the same size as the liver. The amount injected must be small in order to avoid complete arrest of circulation by arterial constriction. With the denervated nictitating membrane and the non-pregnant cat's uterus as test objects, it can be shown that from 75 to 90% of 2 to 5  $\mu\text{g}$  of adrenaline disappear in the hind limb and that the same percentage is inactivated by the liver (72).

6) The argument put forward by Gaddum and Kwiatkowski (66) that ephedrine sensitizes to adrenaline by virtue of its inhibitory action on amine-oxidase does not seem to be valid for at least three reasons: a) ephedrine sensitizes tissues deprived of amine-oxidase (67), b) ephedrine sensitizes in very weak concentrations which *in vitro* do not inhibit amine-oxidase (67), c) cocaine abolishes completely and rapidly the sympathomimetic action of ephedrine, while it simultaneously increases the actions (mainly *excitatory*) of adrenaline (74). A detailed study of the effects of ephedrine and adrenaline on various smooth muscles and under various physiological and pharmacological conditions shows beyond question that ephedrine acts directly on the cells and not indirectly by way of a sensitization to the actions of adrenaline or sympathin. For example, ephedrine normally contracts the chronically denervated nictitating membrane of the anesthetized and even the adrenalectomized cat, thus it acts on a tissue deprived of its store of adrenaline-like substances, in an animal which has no adrenaline or sympathin in the circulation (74). It cannot be argued that cocaine suppresses the action of ephedrine because of its own inhibitory action on amine-oxidase, the fact that cocaine rapidly inhibits the long-lasting contraction of the nictitating membrane caused by the prior injection of ephedrine is not compatible with the hypothesis of Gaddum, supported by Tripod (75) and MacGregor (76).

7) The argument that cocaine and the local anesthetics which sensitize to adrenaline also inhibit amine-oxidase (77) seems at first sight more difficult to refute. But there is no parallelism between the degree of enzyme inhibition and the sensitizing power. For example, nupercaine was found by Philpot (77) to be the best inhibitor of amine-oxidase, yet it potentiates only slightly the action of adrenaline on the nictitating membrane "*in situ*", in contrast procaine, a weaker inhibitor, sensitizes markedly (78).

Furthermore, Philpot's hypothesis does not explain why cocaine, instead of increasing, completely abolishes the action of tyramine and phenylethylamine (74) which are oxidized *in vitro* by amine-oxidase (63, 64).

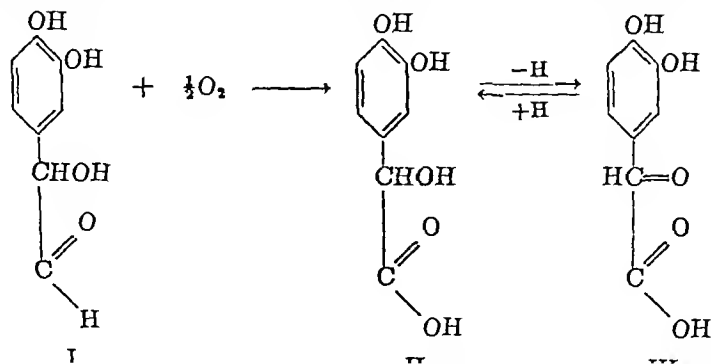
The phenomena of sensitization and desensitization to sympathomimetic compounds are very complex in the case of cocaine and local anesthetics, and at present cannot be interpreted on the basis of a theory of enzyme inhibition.

8) Efficient inhibitors of amine-oxidase such as ethylurethane (54) do not sensitize *in vivo* to adrenaline. Sulfoconjugated adrenaline is found in the urine of man and animals given large doses of adrenaline (80, 86, 87). Although the direct experiment has not been tried, there is *a priori* no reason why amine-oxidase should not deaminate and oxidize the side-chain of the sulfuric ester of adrenaline or epinine.

9) According to Weinstein and Manning (79), the urine of rabbits given large doses of adrenaline shows the reaction for protocatechuic acid  $(\text{OH})_2\text{C}_6\text{H}_3\text{COOH}$  which should be the final product of the oxidation *in vivo* of the aldehyde resulting from amine-oxidase action on adrenaline. Unfortunately, as pointed out by Richter (80) and by Bernheim (1), these authors treated the urine with alkali and adrenaline, if present, would be converted in part to protocatechuic acid. Inasmuch as the presence of free and conjugated adrenaline has been demonstrated in the urine, the evidence brought forward by Weinstein and Manning is not conclusive. Richter (80) tried without success to find, by means of a sensitive color reaction, protocatechuic acid in his own urine after ingestion of large doses of epinine (61 mg) or adrenaline (*d* or *l*, from 10 to 55 mg), even after acid hydrolysis, which should liberate protocatechuic acid from its sulfoconjugate, the test was negative.

Florkin and Bacq (81), employing Baumann's method which avoids the use of alkali, have observed the presence of phenolic acid in the urine of a dog which received 150 mg of epinine by intraperitoneal injection, but epinine is a better substrate for amine-oxidase than is adrenaline, and the amount injected was enormous.

Confirmation of the negative results of Richter is given by the fact that up to 70% of tyramine perfused through the rabbit's liver (63) was recovered as 4-oxyphenylacetic acid. Large quantities of the same acid were found in the urine of dogs and rabbits after large doses of tyramine were given (63, 64). The assumption of Weinstein and Manning that protocatechuic acid  $(\text{OH})_2\text{C}_6\text{H}_3\text{COOH}$  must be the final product of adrenaline oxidation by amine-oxidase is open to question. If one considers the probable fate of dihydroxyphenyl-hydroxyacetaldehyde (I) in the body, it appears that two flavoproteins (xanthine oxidase and a specific aldehyde oxydase) will oxidize it to the corresponding acid (II),



which is the 3,4-hydroxymandelic acid. If this acid follows the metabolism of mandelic acid ( $C_6H_5 \text{ CHO} \text{H COOH}$ ), it should be mainly excreted with the two-carbon side-chain unaltered. It might be partly sulfoconjugated at the level of at least one of the phenolic groups. An equilibrium might also occur between the acid and its ketone (III) (for bibliography, see ref 82, page 115).

It is not probable that the presence of the phenolic hydroxyls alters the fate of the side-chain since para-hydroxyphenylacetic acid,  $OH \text{ C}_6\text{H}_4 \text{ CH}_2 \text{ COOH}$ , passes through the body unchanged (83). This last observation is interesting because it shows that in this particular case the addition of a two-carbon side-chain to the phenol molecule inhibits the sulfoconjugation (see section V). Thus a careful review of the literature does not indicate how 3,4-oxymandelic acid could lose a carbon atom and be changed into protocatechuic acid.

Even if one accepts the view that protocatechuic acid is formed from adrenaline, one should find in the urine not only the sulfuric ester of this acid, but also some free acid and sulfoconjugated catechol. It is known that these three substances are found in the urine of the dog fed with protocatechuic acid (84).

**VI ESTERIFICATION OF THE PHENOLIC GROUPS** The two phenolic hydroxyls of adrenaline are essential in the determination of the quality and the intensity of sympathomimetic action of the aromatic amines. The OH in the *meta* position is more important than the hydroxyl in the *para* position, but only catechol derivatives may be considered as true sympathomimetic amines (see ref 85). Thus oxidation or esterification of these two phenolic groups results in complete inactivation of the molecule, as far as the classical sympathomimetic effects are concerned.

Curiously, it was not before 1940 that the first contribution appeared on the possibility of detoxification of adrenaline by the mechanism of sulfoconjugation, a process so well known for phenolic substances. The evidence given by Richter (80) in favor of this mechanism is the following:

1) Richter ingested large amounts of *d*- or *l*-adrenaline (15 to 55 mg, per 76 kg) with glycine and acetic acid (to avoid, as far as possible, oxidation in the digestive tract). A substance was excreted in the urine which had the characteristics of an adrenaline sulfoconjugate: it was inactive before hydrolysis, after acid hydrolysis, it gave all the chemical reactions (specific and non-specific) of adrenaline\* and inhibited the isolated rabbit's intestine.

2) By the use of a method accurate within 10%, it was shown that the excretion of this conjugated adrenaline began 3 hrs after the ingestion, was maximal in about 5 hrs and then slowly decreased in the course of 24 hrs. The amount of adrenaline recovered as the sulfoconjugate varied from 30 to 70%.

3) An increase in blood pressure indicated that, between 1 and 4 hrs after the ingestion, some active adrenaline reached the tissues, but certainly the greater part of the amine was detoxified in the intestine and the liver.

\* Green color reaction with ferric chloride, formation of adrenochrome and isoadrenochrome, reduction of arsenomolybdic acid with a marked increase in color with NaOH, as described by Shaw (30).

4) Glycuronide tests were negative, tests for sulfuric esters were positive. The sulfoconjugate has not been isolated in pure state, its structure is tentatively given as  $\bar{O}-SO_2 \cdot O (OH) C_6H_3 CHOH CH_2 \overset{+}{N}HCH_3$ .

5) Similar observations were made by Richter after ingestion of *dl*-corbasil,  $(OH)_2C_6H_3 CHOH CH (NH_2) CH_3$ , and epinine,  $(OH)_2C_6H_3 CH_2 CH_2 NH-CH_3$ . Confirmation of Richter's observations has been provided by Richter and MacIntosh (86). After hydrolysis, adrenaline has been adequately identified pharmacologically, but only 39% of the ingested adrenaline was accounted for. Beyer and Shapiro (87) have also confirmed Richter's observations and added interesting facts. In dogs, 15.9 and 22.3% of 25 mg of ingested adrenaline were recovered in 8 hours as the sulfoconjugate. A much higher amount (50 to 78%) of epinine was recovered in this form in 8 hours and the percentage rose to 83% when the urine was collected for 24 hours. If 30 mg of epinine were injected subcutaneously in dogs, 40 to 50% was recovered in 8 hours as the sulfoconjugate, after ingestion of an equal amount, 50 to 79% was recovered in the same period. An average of 65% of ingested cobefrine (3,4-dihydroxyphenylisopropanolamine), which is not oxidized by amine-oxidase, was recovered in 24 hours as the sulfoconjugate.

Florkin and Bacq (81), quite independently from Richter, tried to find evidence for the excretion of the sulfoconjugate with a different technique. In dogs weighing 6 to 7 kg, 100 mg of catechol (injected subcutaneously or intraperitoneally) are necessary to obtain a decrease below unity of the ratio  $\frac{\text{inorganic S}}{\text{ester S}}$  in the urine collected during the 24 hrs following the injection. A dose of 50 mg does not provoke the so-called "reversal" of the ratio. If equimolecular amounts of adrenalone (the ketone of adrenaline) or epinine are injected intraperitoneally in these dogs, the ratio  $\frac{\text{inorganic S}}{\text{ester S}}$  remains unchanged.

It cannot be concluded from these experiments that adrenalone and epinine are not esterified. Part of these catechol derivatives (50% or less) still may be detoxified by sulfoconjugation. The presence of an aminated side-chain has undoubtedly modified the metabolism of the catechol nucleus.<sup>4</sup> A more extensive use of Florkin and Bacq's method might show with fair approximation what proportion of the amines derived from catechol is excreted as sulfoconjugate, but, at first sight, these results do not contradict the observations of Richter and of Beyer and Shapiro.

Deichmann (88) has observed no increase in glycuronides, but a marked decrease of the ratio inorganic/total sulfates in the urine of rabbits following oral, subcutaneous or intravenous administration of adrenaline. Some of his results are in contradiction with the carefully controlled observations of Florkin and Bacq (81), and a far greater quantity of organic sulfates was excreted than could be accounted for by the conjugation of the administered adrenaline. Dogson,

<sup>4</sup> It has already been mentioned (page 8) that para-oxyphenylacetic acid,  $HO-C_6H_4-CH_2-COOH$ , is excreted unchanged in the urine.

Garton and Williams (201) failed to confirm Deichmann's observations, in rabbits given 200 to 250 mg/kg of *d*-adrenaline orally, they found insignificant amounts of sulfoconjugate in the urine, whereas 21% was excreted as a glucuronide in 24 hours. The question whether *l*-adrenaline also forms a glucuronide has yet to be investigated because *l*-adrenaline suppresses glucuronic acid conjugation in liver slices (202).

The great objection to all these experiments is that very large, unphysiological amounts of sympathomimetic compounds are needed. One cannot extrapolate from these results what happens to a few micrograms of adrenaline in the arterial blood. A further objection to Richter's work is that the adrenaline was taken orally and consequently only a very small fraction reached the heart and the general circulation.

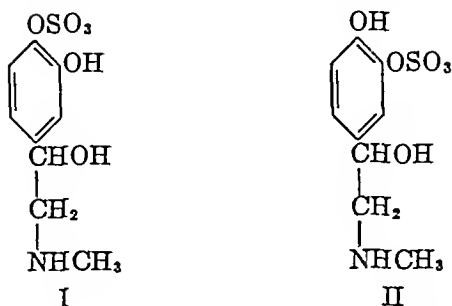
Although the ability of the liver and intestine to detoxify adrenaline is definitely established, it is beyond question (in contrast to common belief) that the liver is not the main site of detoxification of adrenaline circulating in physiological concentrations. The experiments of Bacq (72) show that, if one uses very small amounts (2 to 5  $\mu$ g), the cat's hind limb can inactivate as much adrenaline as the liver (see also ref. 5).

The enzymatic system called sulfosynthase which catalyzes the esterification of phenolic substances seems to be localized in the liver and in the intestinal mucosa (89, 90, 104), although it has been reported that eviscerated animals still conjugate phenols (91). It has already been mentioned that evisceration does not increase or prolong the action of adrenaline injected in physiological amounts, thus it seems that the action of this liver sulfosynthase is not a predominant factor in the physiological inactivation of adrenaline.

Bernheim (1) points out that Richter's observations leave certain important facts unexplained. "Esterification does not require oxygen, yet perfused liver does not inactivate adrenaline unless oxygen is present and the same is true of isolated liver slices." It appears, however, that the conjugation requires the energy produced by reactions coupled with oxygen consumption (87). The adrenaline ester recovered from the human urine is pharmacologically inactive in small doses (86), from what we know of the relation between chemical structure and physiological activity in the series of sympathomimetic amines, "it is also not entirely clear why esterification of one hydroxyl group should make adrenaline pharmacologically inactive, even though the sulfate ester of tyramine has been reported to be inactive in the rabbit" (92).

Trimethoxyphenylethylamine (mescaline) at a dose of 10 mg/kg is not sympathomimetically inactive (74), the monosulfuric ester of adrenaline still possesses a phenolic hydroxyl which is known to increase (markedly, if in the *meta* position) the action of aromatic amines. Of the two possible isomers of Richter's ester, ester II should be less active than I, but not completely inactive in large doses.

The suggestion that esterification of adrenaline occurs only if the amine is ingested has not been supported by the experiments of Bacq, Lecomte and Fischer (93). In the urine of chloralosed dogs given intravenous injections of large



amounts of adrenaline, these investigators found an inactive substance which gave the physiological actions of adrenaline after acid hydrolysis, it was presumably the same sulfuric ester as that reported by Richter. The percentage recovered in 8 hours was about 5%. The only objection remaining is that the quantity injected (5 to 10 mg) was unphysiological. With the available physiological and biochemical techniques, it would appear possible to make conclusive observations with smaller amounts of adrenaline.

Holtz *et al* (94), in confirmation of earlier observations, found a pressor substance "urosympathin" in the urine of normal man, subjected to acid hydrolysis, a closer analysis showed that this urosympathin is a mixture of adrenaline and its two postulated precursors arterenol and oxytyramine. The daily excretion, as tested on the cat's blood pressure, was equivalent to 0.1–0.15 mg of adrenaline or 2–3 mg of oxytyramine. The excretion of urosympathin is increased by muscular work and in some cases of arterial hypertension (see also ref 2). Although Holtz *et al* do not mention the fact, their urosympathin is probably in the urine as an inactive sulfoconjugate. Richter (80) and those who have confirmed his views in men and animals, did not find a sulfoconjugate of a sympathomimetic amine in normal urine, but the small amount normally present may have escaped their attention.

Torda (95) attempted to explain the actions of cocaine, ergotamine and yohimbine by their effect *in vitro* on a very weak preparation of liver sulfo-esterase, for many reasons, Bernheim (1) is correct in his statement that these experiments are of little value, although cocaine increases slightly the excretion of free phenol in the cat after phenol injection (96).

Experiments of Bacq (97) have shown that many phenols sensitize markedly to adrenaline and to sympathetic stimulation. This sensitization, quite different from that following cocaine injection, had been related to the well-known antioxidant power of these unstable phenols (catechol, hydroquinone, pyrogallol) and it had been considered as an argument in favor of the oxidation (through adrenochrome) of adrenaline *in vivo*. Richter (80) suggests that these phenols compete with adrenaline for the inactivating system and act to augment and prolong the effects of adrenaline by inhibiting its sulfoconjugation, he draws an analogy between the inhibition of cholinesterase by eserine and the inhibition of "sulfo-synthase" by phenols. There is the following objection to Richter's interpretation: resorcinol, which is sulfoconjugated as readily as catechol or



Garton and Williams (201) failed to confirm Deichmann's observations, in rabbits given 200 to 250 mg/kg of *d*-adrenaline orally, they found insignificant amounts of sulfoconjugate in the urine, whereas 21% was excreted as a glucuronide in 24 hours. The question whether *l*-adrenaline also forms a glucuronide has yet to be investigated because *l*-adrenaline suppresses glucuronic acid conjugation in liver slices (202).

The great objection to all these experiments is that very large, unphysiological amounts of sympathomimetic compounds are needed. One cannot extrapolate from these results what happens to a few micrograms of adrenaline in the arterial blood. A further objection to Richter's work is that the adrenaline was taken orally and consequently only a very small fraction reached the heart and the general circulation.

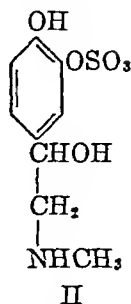
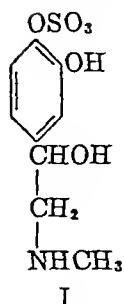
Although the ability of the liver and intestine to detoxify adrenaline is definitely established, it is beyond question (in contrast to common belief) that the liver is not the main site of detoxification of adrenaline circulating in physiological concentrations. The experiments of Bacq (72) show that, if one uses very small amounts (2 to 5  $\mu$ g), the cat's hind limb can inactivate as much adrenaline as the liver (see also ref. 5).

The enzymatic system called sulfosynthase which catalyzes the esterification of phenolic substances seems to be localized in the liver and in the intestinal mucosa (89, 90, 104), although it has been reported that eviscerated animals still conjugate phenols (91). It has already been mentioned that evisceration does not increase or prolong the action of adrenaline injected in physiological amounts, thus it seems that the action of this liver sulfosynthase is not a predominant factor in the physiological inactivation of adrenaline.

Bernheim (1) points out that Richter's observations leave certain important facts unexplained. "Esterification does not require oxygen, yet perfused liver does not inactivate adrenaline unless oxygen is present and the same is true of isolated liver slices." It appears, however, that the conjugation requires the energy produced by reactions coupled with oxygen consumption (87). The adrenaline ester recovered from the human urine is pharmacologically inactive in small doses (86), from what we know of the relation between chemical structure and physiological activity in the series of sympathomimetic amines, "it is also not entirely clear why esterification of one hydroxyl group should make adrenaline pharmacologically inactive, even though the sulfate ester of tyramine has been reported to be inactive in the rabbit" (92).

Trimethoxyphenylethylamine (mescaline) at a dose of 10 mg/kg is not sympathomimetically inactive (74), the monosulfuric ester of adrenaline still possesses a phenolic hydroxyl which is known to increase (markedly, if in the *meta* position) the action of aromatic amines. Of the two possible isomers of Richter's ester, ester II should be less active than I, but not completely inactive in large doses.

The suggestion that esterification of adrenaline occurs only if the amine is ingested has not been supported by the experiments of Bacq, Lecomte and Fischer (93). In the urine of chloralosed dogs given intravenous injections of large



amounts of adrenaline, these investigators found an inactive substance which gave the physiological actions of adrenaline after acid hydrolysis, it was presumably the same sulfuric ester as that reported by Richter. The percentage recovered in 8 hours was about 5%. The only objection remaining is that the quantity injected (5 to 10 mg) was unphysiological. With the available physiological and biochemical techniques, it would appear possible to make conclusive observations with smaller amounts of adrenaline.

Holtz *et al* (94), in confirmation of earlier observations, found a pressor substance "urosympathin" in the urine of normal man, subjected to acid hydrolysis, a closer analysis showed that this urosympathin is a mixture of adrenaline and its two postulated precursors arterenol and oxytyramine. The daily excretion, as tested on the cat's blood pressure, was equivalent to 0.1-0.15 mg of adrenaline or 2-3 mg of oxytyramine. The excretion of urosympathin is increased by muscular work and in some cases of arterial hypertension (see also ref 2). Although Holtz *et al* do not mention the fact, their urosympathin is probably in the urine as an inactive sulfoconjugate. Richter (80) and those who have confirmed his views in men and animals, did not find a sulfoconjugate of a sympathomimetic amine in normal urine, but the small amount normally present may have escaped their attention.

Torda (95) attempted to explain the actions of cocaine, ergotamine and yohimbine by their effect *in vitro* on a very weak preparation of liver sulfo-esterase, for many reasons, Bernheim (1) is correct in his statement that these experiments are of little value, although cocaine increases slightly the excretion of free phenol in the cat after phenol injection (96).

Experiments of Bacq (97) have shown that many phenols sensitize markedly to adrenaline and to sympathetic stimulation. This sensitization, quite different from that following cocaine injection, had been related to the well-known antioxidant power of these unstable phenols (catechol, hydroquinone, pyrogallol) and it had been considered as an argument in favor of the oxidation (through adrenochrome) of adrenaline *in vivo*. Richter (80) suggests that these phenols compete with adrenaline for the inactivating system and act to augment and prolong the effects of adrenaline by inhibiting its sulfoconjugation, he draws an analogy between the inhibition of cholinesterase by eserine and the inhibition of "sulfosynthase" by phenols. There is the following objection to Richter's interpretation: resorcinol, which is sulfoconjugated as readily as catechol or

pyrogallol but which is not an antioxidant because it is stable, does not sensitize to adrenaline even if injected in an amount three times that of catechol (97)

Thus substantial amounts of inactive sulfoconjugated catecholamines are found in the urine of dog and man after oral ingestion or subcutaneous or intravenous injections. After injection, the amounts recovered are less than after ingestion. Quantitative data indicate that the sulfoconjugation of the catecholamines is not as complete as that of catechol, and other possible pathways of adrenaline inactivation must be sought. This conclusion is consistent with the fact that adrenaline disappears in tissues which are not known to conjugate phenols.

**VII OXIDATION TO QUINONE** *A Introduction* *In vitro*, aqueous solutions of adrenaline are rapidly oxidized by molecular oxygen with the formation of a red color. This oxidation is accelerated by the presence of traces of copper (Fe, Hg and other heavy metals), by an alkaline pH and by light (ultraviolet rays). Many substances inhibit this oxidation, for example, strong reducing agents such as glutathione (103), cysteine, ascorbic acid (26, 103) and dimercaptopropanol (BAL) (27), amino acids (98, 99), the so-called "antioxygens," such as phenols and thyroxine (97, 100). The presence of some of these substances in the blood and the tissues has undoubtedly a physiological significance. In the adrenals of mammals and in the parotoid glands of tropical toads where adrenaline is concentrated without any tendency to oxidize, one finds large concentrations of ascorbic acid and glutathione.

Thyroxine is beyond question one of the most important chemical agents which normally regulate the sensitivity of the tissues to adrenaline (48, 101, 102, 97). Physiologists and pharmacologists are aware of the fact that, when secretion of adrenaline is considered, not only is the quantity secreted important but also the sensitivity of the effector cells, which may vary considerably (for discussion, see ref. 97). The so-called "sympathetic hyperexcitation" state of hyperthyroid patients is probably a purely peripheral phenomenon. It seems probable that these variations in the sensitivity to adrenaline are due to changes in the metabolism of the hormone. For example, after pyrogallol injection, the denervated nictitating membrane reacts to adrenaline by a long-lasting contracture similar to that obtained with epinephrine in the normal cat, although adrenaline disappears from the blood (49).

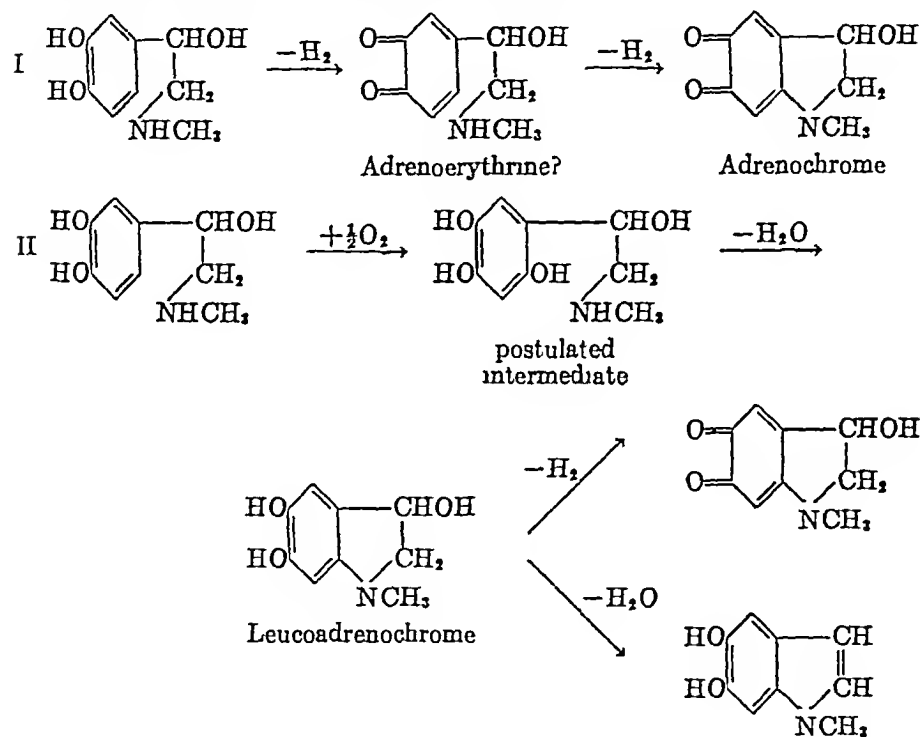
Before discussing the properties of adrenochrome, three questions should be answered.

1) Is the slow oxidation *in vitro* (autooxidation) comparable to the oxidation by catecholoxidase? The answer is presumably yes. Oxidation of adrenaline by silver oxide gives the same adrenochrome as that by catecholoxidase. The catalytic action of  $\text{Cu}^{++}$  ions is known to be similar to that of the phenolases, the active metal of which is copper.

One should not too strongly emphasize the fact that a red solution of autooxidized adrenaline cannot be considered as adrenochrome, because adrenochrome is very unstable and is rapidly transformed into many different substances. The

complete oxidation of adrenaline to melanin requires 8 to 9 atoms of oxygen per molecule of adrenaline, and some  $\text{CO}_2$  is evolved (103), only 2 atoms of oxygen are needed to obtain adrenochrome. A careful review of the literature shows that several melanins requiring different amounts of oxygen may be obtained from adrenaline.

2) Is it possible to conceive the existence of a substance intermediate between adrenaline and adrenochrome? Schematically, two mechanisms are possible to account for the transition from adrenaline to adrenochrome. In sequence I, the phenolic groups are first oxidized, the side-chain being open, in sequence II, the side-chain reacts prior to the oxidation of the hydroxy groups.



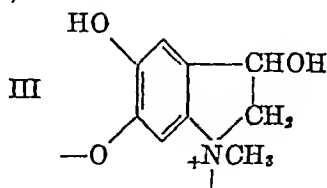
These two possibilities have been much discussed (105, 106, 107, 108, 109), it is not within the scope of this article to enter into the details of the chemical reactions.

If sequence I is correct, one should expect the quinone with open chain to retain some sympathomimetic activity and to yield adrenaline on reduction. Many authors (110, 111, 112, 113, 26) have published observations in favor of the existence of this adrenaline quinone or "adrenorythrine" (26), but the substance has never been isolated (not even in the form of stable derivatives such as the oxime or semicarbazone).

If sequence II is correct, reduced adrenochrome, the so-called leucoadrenochrome (107, 114), is produced before adrenochrome itself. If we accept Harley-

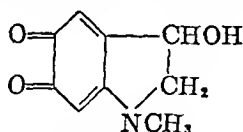
Mason's view (114), this series of reactions is impossible because the very unstable leuco derivative easily loses a molecule of water, and one should never obtain the quinone adrenochrome

The possibility of the existence of a semiquinone (type III) has been emphasized only recently (114)

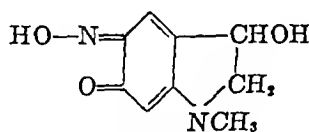


3) Does "autoxidation" of adrenaline occur in the body? The answer is that this is quite unlikely, as pointed out by Richter (80), because the cells contain many substances which stabilize adrenaline

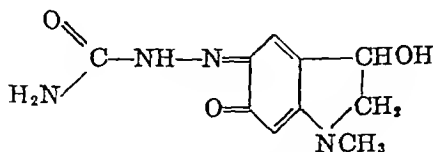
*B Chemical properties and derivatives of adrenochrome* A very important contribution by Green and Richter was the isolation and clear identification of the quinone resulting from adrenaline oxidation (107). They used a highly purified catecholoxidase from mushroom, a concentrated solution ( $1 \times 10^{-1}$ ) of adrenaline, a pH of 5 to prevent melanin formation, and bubbling  $O_2$ . Adrenochrome is now prepared more easily by the action of silver oxide on adrenaline in methyl alcohol (115, 116). Adrenochrome is very unstable even when crystallized in the cold, in the dark and in the absence of  $O_2$ . It turns to a brownish black, insoluble pigment which is by definition a melanin. Three stable derivatives of adrenochrome have been isolated: the monoxime (107, 115, 116), the mono-semicarbazone<sup>6</sup> (116) and the mono-*p*-nitrophenylhydrazone (115), by reacting one of the quinone oxygens with hydroxylamine, semicarbazide or *p*-nitrophenylhydrazine, respectively



Adrenochrome  
(according to 107)



Monoxime

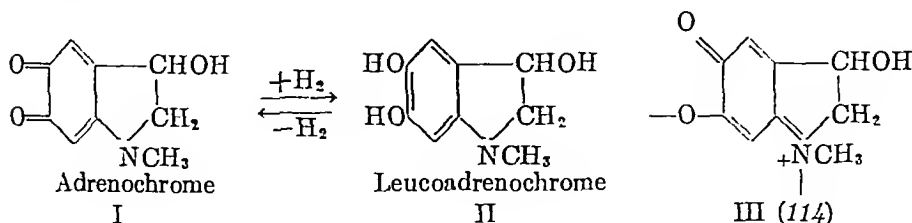


Mono-semicarbazone  
(Adrenovyl)

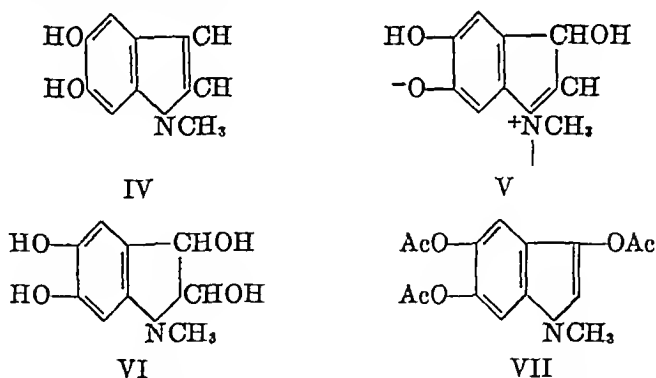
In the presence of a strong reducing agent (hydrosulfite, ascorbic acid, cysteine, Raney's nickel), the red color of adrenochrome changes to various shades of light

<sup>6</sup> Also known in Europe as "Adrenoxyl Labaz" Société belge de l'Azote

green, presumably the color of the assumed leucoadrenochrome which has not been isolated. There seems to be a redox equilibrium between the two forms (I and II)



Recent investigations show that the physical and chemical properties of adrenochrome are better accounted for by the structure of a zwitterion, paraquinoneimine (III) (114). For example, the facts that only the *monoxime* and the *mono-semicarbazone* have been prepared and that all attempts to induce a reaction with a second molecule of hydroxylamine or semicarbazide have been unsuccessful show that only one true carbonyl group is present in the molecule of adrenochrome. A careful study of the reduction with hydrogen-Pd-charcoal shows that absorption of hydrogen ceases when one atom of hydrogen per molecule of adrenochrome has been absorbed<sup>6</sup> and two products have been obtained, none of them has the supposed structure of leucoadrenochrome (II). One of these substances is the 5,6-dihydroxy-N-methyl-indole (IV) which has been isolated in colorless needles

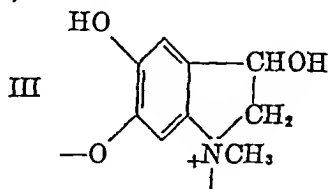


The other (V) could not be isolated as such because it decomposes on concentration, but two stable derivatives were obtained VI after treatment with alkali and VII after acetylation. The compound VI is yellow and exhibits a strong fluorescence in ultraviolet light, it is probably responsible for the color appearing in the test of Gaddum and Schild (117), the intensity of which is specific for adrenaline in the series of sympathomimetic amines, these authors observed a marked green fluorescence when adrenaline is oxidized (for example, at an

<sup>6</sup> Unpublished observations by Beaudet show that the same absorption of a single H atom occurs when adrenochrome is reduced by Raney's nickel.

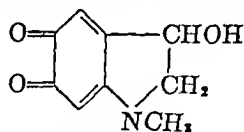
Mason's view (114), this series of reactions is impossible because the very unstable leuco derivative easily loses a molecule of water, and one should never obtain the quinone adrenochrome

The possibility of the existence of a semiquinone (type III) has been emphasized only recently (114)

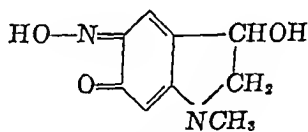


3) Does "autoxidation" of adrenaline occur in the body? The answer is that this is quite unlikely, as pointed out by Richter (80), because the cells contain many substances which stabilize adrenaline

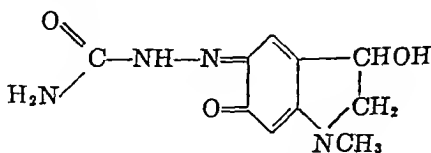
*B Chemical properties and derivatives of adrenochrome* A very important contribution by Green and Richter was the isolation and clear identification of the quinone resulting from adrenaline oxidation (107). They used a highly purified catecholoxidase from mushroom, a concentrated solution ( $1 \times 10^{-2}$ ) of adrenaline, a pH of 5 to prevent melanin formation, and bubbling  $O_2$ . Adrenochrome is now prepared more easily by the action of silver oxide on adrenaline in methyl alcohol (115, 116). Adrenochrome is very unstable even when crystallized in the cold, in the dark and in the absence of  $O_2$ . It turns to a brownish black, insoluble pigment which is by definition a melanin. Three stable derivatives of adrenochrome have been isolated: the monoxime (107, 115, 116), the mono-semicarbazone<sup>5</sup> (116) and the mono-*p*-nitrophenylhydrazone (115), by reacting one of the quinone oxygens with hydroxylamine, semicarbazide or para-nitrophenylhydrazine, respectively.



Adrenochrome  
(according to 107)



Monoxime



Mono-semicarbazone  
(Adrenoxyl)

In the presence of a strong reducing agent (hydrosulfite, ascorbic acid, cysteine, Raney's nickel), the red color of adrenochrome changes to various shades of light

<sup>5</sup> Also known in Europe as "Adrenoxyl Labaz" Société belge de l'Azote

Adrenochrome and its oxime and semicarbazone are excellent hemostatic substances as far as capillary hemorrhages are concerned (126, 127, 128, 129), the semicarbazone (*adrenoxyl*) has been widely used with success in Belgium and France in human therapeutics. The lack of toxicity and the absence of any interfering sympathomimetic action are obvious advantages (130). Large amounts of the semicarbazone have weak nicotinic actions (131). They increase capillary resistance (vitamin P action) and this increase is parallel to the hemostatic action (132, 133), iodo-adrenochrome also increases capillary resistance (134). The action of the many substances possessing vitamin P properties has been interpreted as a sensitization phenomenon related to the adrenalinic-adrenochrome action (135).

The hemostatic action of minute amounts of adrenaline (1  $\mu$ g in the rabbit) has a latent period of four minutes, is maximal after 7 minutes and lasts for hours, these facts led Roskam and Derouaux to suspect that some oxidized derivative might be responsible (126, 136). Adrenochrome is as good a hemostatic as adrenaline and its action is maximal three minutes after injection (136). Thus the hypothesis seems justified that this particular long-lasting action of adrenaline is due to adrenochrome or the products of adrenochrome metabolism in the body.

Adrenochrome possesses many properties of the other quinones. It oxidizes reversibly the —SH groups of glutathione, proteins and enzymes, and by this mechanism inhibits many enzymes of the glycolytic cycle: hexokinase, phosphohexokinase, etc (137, 138). The physiological expression of this inhibition is the Lundsgaard contracture (contracture and inexcitability after work) observed in amphibian (139) and mammalian (140) muscles. Like many quinones, adrenochrome inhibits mitosis (141, 142), and the literature shows an increasing tendency to link anti-mitotic action with inhibition of carbohydrate metabolism (175).

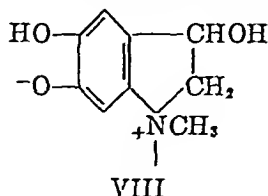
Thus adrenochrome, by virtue of its quinone function or functions, joins the vast group of "thioloprive" substances, a term created by Bacq (143) in order to designate toxic substances of widely different chemical structure which deprive the tissues of their thiol groups, inhibit the same enzymatic systems and possess common pharmacological properties. An important question is whether adrenochrome is one of the chemical factors which control the mitotic activity of cells *in situ*.

The semicarbazone, which is not in itself a thioloprive substance, seems to be hydrolyzed by the tissues and to liberate adrenochrome (139). Adrenoxyl has the unexplained ability to increase, sometimes markedly, the response of the isolated rat's diaphragm to maximal phrenic nerve stimulation (140), this potentiation is not exhibited by adrenochrome and is quite different from the Orbeli effect of adrenaline (140).

Adrenochrome like other quinones catalyzes the inactivation of catecholamines and may play a role in chronic hypertension (144, 145), this last observation has not been confirmed. There is a controversy concerning the action of adrenochrome, its derivatives or unidentified oxidation products of adrenaline on blood sugar, some investigators obtained hyperglycemia, many observed hypoglycemia and an increased effect of insulin (146, 147) and others were unable to find any change (148, 149). Adrenochrome increases glycogen formation (150, 147) and



alkaline pH) According to Utevsky (112), the fluorescence of oxidized adrenaline is due to the supposed leucoadrenochrome (II), this contention seems to be untenable in view of the observations of Harley-Mason (114) Substance IV results from the immediate, irreversible dehydration of the so-called leucoadrenochrome (II) and the redox equilibrium postulated should be impossible Substance V is isomeric with adrenochrome The existence of a common intermediate for substances IV and V is postulated, it should be the semiquinone VIII



If one accepts these views, how is it possible to explain the action of adrenochrome as a hydrogen carrier in the aerobic, malic and lactic dehydrogenase systems (107)? The opinion of Harley-Mason is that, since compounds IV and V cannot be reconverted to adrenochrome, "the redox system involves one electron transfer between adrenochrome and the semiquinone (VIII)" The short note of Harley-Mason does not answer several questions,<sup>7</sup> one must wait for the complete report

Adrenochrome and its derivatives are certainly the most interesting oxidized derivatives of adrenaline It is a great error to consider adrenochrome as an inactive substance because it has lost all its classical sympathomimetic actions (107, 77, 119) Beyer (2) discusses at length the inactivation of sympathomimetic amines in his 1946 review but simply ignores adrenochrome

*C Physiological properties of adrenochrome and its stable derivatives* Adrenochrome was isolated by Green and Richter, it was found to play the role of an efficient hydrogen carrier, a property common to many quinoid substances (122, 123) If adrenaline is added to a lactic acid or malic acid dehydrogenase system, there is a latent period for its oxidation to adrenochrome A measurable effect is obtained even with concentrations as low as  $6 \times 10^{-7}$  "The general experience with oxygen carriers such as cytochrome and lactoflavine is that far higher concentrations are required to obtain an oxygen uptake measurable *in vitro* than are necessary in the living cell It can therefore be concluded that the carrier action of adrenochrome may come within the range of physiological concentrations" (Green and Richter, 107, p 615) Recent work indicates that the adrenaline-adrenochrome system is present in mammalian skeletal muscle at a concentration of approximately  $1 \times 10^{-7}$  (125) Older observations by Kisch and collaborators (120) had shown that oxidized adrenaline (the so-called "omega substance" which is probably adrenochrome and various unidentified substances beyond adrenochrome) catalyzes the oxidation of certain amino-acids and increases oxygen consumption of isolated tissues Blix (124) has also found an action of oxidized adrenaline on amino-acids

<sup>7</sup> For example, it is difficult to understand how substance VI is colored when IV is colorless

the amount recovered does not exceed 25% (158) The fate of adrenochrome in the body needs further investigation

When adrenoxyl (semicarbazone of adrenochrome) is orally administered to fasting persons, a large part (20 to 25%) is excreted unchanged by the kidneys, there is an increased excretion of indolic substances, but this seems to be due to the activity of intestinal bacteria because it does not occur if a sulfonamide has been ingested in adequate amounts (158) When injected in dogs and rabbits, adrenoxyl is rapidly excreted unchanged in the urine, but there is also an increased excretion of other indolic substances (158) When *dl*-adrenaline is injected in large, anesthetized dogs (subsequent to the injection of an adrenolytic substance such as 933F or yohimbine), there is an increased excretion of indolic substances equivalent to 10-20% of the adrenaline injected This increased excretion of indolic substances roughly parallels the excretion of the adrenaline sulfoconjugate One may argue that the large amounts of adrenaline injected are unphysiological (0.5 to 20 mg), and that an increased protein catabolism may result in the excretion of larger amounts of indolic substances from tryptophane or tyrosine However, simultaneous determinations of urinary urea show that this second objection is not valid (23)

*In vitro*, the oxidation of adrenaline to adrenochrome is catalyzed not only by catecholoxylase, the existence of which is questionable in mammalian tissues,<sup>8</sup> but also by the cytochrome-indophenol-oxidase system present in all tissues (107, 162, 164) and by a cyanide-insensitive enzymatic system present in the heart and skeletal muscles (107) Green and Richter (107) have probably been dealing with the same powerful enzymatic system described by Schütz in liver extracts (165) There is no reason to suppose that these enzymes are not active *in vivo*, and the increased excretion of indolic substances after adrenaline injection may be considered as the result of their activity The combination of the rapid disappearance of small amounts of adrenaline in all tissues and of their long-lasting hemostatic action is thus satisfactorily explained Clark and Raventos (167) observed a prolonged action of adrenaline on the frog's auricle after the administration of ascorbic acid The heart muscle contains a high concentration of the cytochrome system which is inhibited by ascorbic acid (164)<sup>9</sup>

Bennet and Hausberger (166) have elegantly demonstrated that adrenaline is

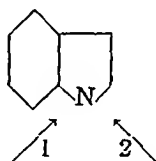
<sup>8</sup> The literature on this point is confusing, many authors claim that there is no catecholoxylase in mammalian tissues (160, 161), but some believe the contrary (162) The experiments showing that tissue extracts or slices oxidize adrenaline with production of a black pigment do not prove the presence of a catecholoxylase because 1) adrenochrome may give melanin without the aid of an enzymatic system and 2) there are other enzymes which oxidize adrenaline to adrenochrome (163, 107)

<sup>9</sup> Beyer and Shapiro (87) do not believe that the cytochrome system oxidizes adrenaline *in vivo*, although the fact is demonstrated *in vitro* Their main argument is that "hydroquinone, catechol and homogentisic acid, which are also oxidized readily by cytochrome *in vitro*, escape this oxidation *in vivo* and appear in the urine as such or in conjugated forms" But this fact does not exclude the possibility that a certain fraction of catechol is oxidized to quinone, catechol and other phenols have the same antimutagenic action as the quinones and it is difficult to give an interpretation of this fact without assuming the formation of a certain amount of quinone in the body from these phenols (204) The possibility of several simultaneous metabolic pathways of the phenylamines must not be overlooked

induces the formation of verdohaemochromogen from haemochromogen (115) Oxidized adrenaline also catalyzes the oxidation of glyceraldehyde (200) The action of adrenochrome on the circulation is discussed by Marquardt and Oettel (203)

*D Adrenochrome as mother-substance of sympathin* The theory of chemical transmitters postulates the existence, at the endings of adrenergic nerves, of pre-formed sympathin easily liberated by the nerve impulse It cannot be questioned that this store exists, but the problem arises whether adrenochrome can be resynthesized to the active amine, in other words, is adrenochrome the "mother-substance" of sympathin?

The perfusion with saline solution of the isolated amphibian heart (33, 151) or the rabbit's ear (136), combined with frequent stimulation of the corresponding adrenergic nerves, results in the progressive disappearance of the cardioaccelerator and vasoconstrictor effects This is interpreted as being due to the loss of the peripheral store of sympathin or of its precursor If small amounts of adrenochrome or oxidized "inactive" adrenaline are added to the perfusion fluid, the normal effects of nerve stimulation are re-established Does this mean that adrenochrome is the precursor of adrenaline? *In vitro* as well as *in vivo*, not a single observation exists showing that indole or a molecule with an indole nucleus breaks between the nitrogen and the nucleus, as shown by arrow 1, when it does break, it is at the position shown by arrow 2 (152) Thus, chemically speaking, the oxidation of adrenaline to adrenochrome is irreversible (see also ref 114)



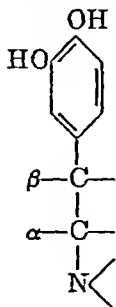
The synthesis of adrenaline proceeds from phenylalanine to dioxypheylalanine, then to oxytyramine by decarboxylation, arterenol (noradrenaline) probably being the immediate precursor of adrenaline (153-157, 10)

*E Occurrence and metabolism of adrenochrome in the body* The presence of adrenochrome in mammalian tissues or blood has never been unequivocally established The observations of Green and Richter and of Roskam and Derouaux already referred to provide only indirect evidence One may hope that, as a result of Harley-Mason's recent contribution, specific and accurate methods will be developed for the quantitative detection of adrenochrome and its derivatives in tissue extracts

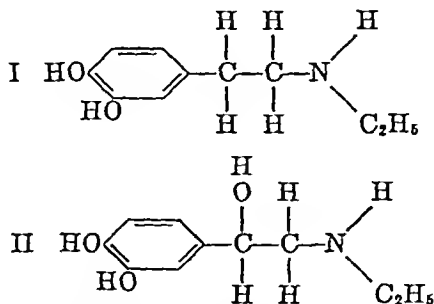
The argument of Richter (80) that after adrenaline is ingested the urine never gives the reactions of adrenochrome has little weight When large doses of adrenochrome (up to 18 mg/kg) are injected subcutaneously into rabbits, adrenochrome is not excreted in the urine and there is no increase in the excretion of indolic substances titratable with the ninhydrin reaction When injected intravenously in dogs, adrenochrome (50 to 100 mg of pure crystallized substance) is excreted in the urine, this excretion ends after about 45 minutes and

amine, for example) abolish the inhibitory effects of adrenaline, but do not inhibit the excitatory actions (58, 176)

2) In the series of the catechol derivatives, there is a subtle correlation between chemical structure and intensity of inhibitory action Barger and Dale (183) had demonstrated that the presence of a single methyl group on the nitrogen is a



determinant factor for true inhibitory activity (for example, on the non-pregnant cat's uterus, the pure test for inhibition<sup>11</sup>), noradrenaline (without a methyl group) and methedrine (with two  $-\text{CH}_3$  radicals) have little inhibitory power. Recent investigations have confirmed this law, but the importance of hydroxyl group on the  $\beta$ -carbon of the side-chain has also been stressed (185, 186). The best demonstration is the following: N-ethoxytyramine (I) is about twenty times less active than adrenaline on the basis of tests for excitation (nictitating membrane, blood pressure) and has almost no inhibitory actions (non-pregnant cat's uterus, blood pressure after 933 F), on the contrary, N-ethylarterenol (II), which also is much less active than adrenaline in the test for excitation, has *qualitatively* and *quantitatively* the same powerful inhibitory effects as adrenaline (186)



This must be correlated with differences in their chemical behavior: arterenol (very weak inhibitor) does not oxidize to an "arterenolochrome" corresponding

<sup>11</sup> It might be useful to call attention to the fact that the isolated mammalian or birds' intestine is not a pure test for inhibition because adrenaline acts on the neurones which regulate the automatism of this smooth muscle (184). A great deal of the discrepancies found in the literature as regards the inhibitory action of sympathomimetic amines is due to the use of the intestine as the test for inhibition. Cushny, Dale and Cannon have always chosen for this purpose the faithful uterus of the non-pregnant cat.

the substrate for melanin formation in the iris. In the young rabbit, sympathetic denervation of the eye results in the lack of pigmentation, thus, one can reproduce experimentally the heterochromia known in human patients to be associated with congenital absence of sympathetic innervation. If adrenaline solutions are instilled in the conjunctival sac, one succeeds not only in producing normal pigmentation but sometimes hyperpigmentation. Thus it is the adrenaline (or adrenaline-like substance) constantly liberated in the iris by the adrenergic nerves which is the substrate for the phenolase of that organ. To be sure, only a negligible fraction of adrenaline is metabolized to melanin, albino rabbits exhibit the same response and sensitivity to adrenaline as do black rabbits (168).

*F. Oxidized derivatives, beyond adrenochrome, obtained in vitro.* With biological tests, one can obtain the most contradictory results, because solutions of more or less "oxidized adrenaline" are mixtures of many substances in variable proportions. The best demonstration of this basic fact is the following. If one oxidizes a  $1 \times 10^{-4}$  solution of adrenaline at room temperature and neutral pH with a catecholoxidase, one obtains precipitate of melanin at a time when the red, clear solution still gives the physiological effects of adrenaline. Thus, in this solution all the intermediates are present together, with some of the unaltered substrate and the end-product. Under well-defined conditions, weaker solutions oxidize more uniformly.

Some authors obtained acetylcholine-like effects<sup>10</sup> with oxidized derivatives of adrenochrome (170, 171), but if one adds cysteine these effects are inhibited. Fresh solutions of adrenochrome and cysteine should have an adrenaline-like action. Gijon (172) states that adrenaline solutions which are autooxidizing *in vitro* have a powerful contracting effect on isolated uterus, and that this effect is inhibited by adrenaline.

The pertinent literature is very confusing and it may be hoped that Harley's recent contribution will clarify the subject. Chemically speaking, it is possible that the secondary alcohol group is oxidized to an "oxoadrenochrome" which has not been isolated (173), it has been known for a long time that *L*-adrenaline oxidizing at an alkaline pH loses its optical activity (165) which adrenochrome still keeps (107). Melanin should be a polymer of this oxoadrenochrome (174).

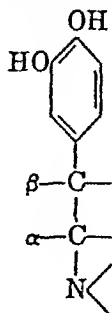
A series of investigations by Bacq and Heirman has linked the inhibitory action of adrenaline with the production of a powerful inhibitory substance beyond adrenochrome (58, 177, 178, 179, 180, 181, 182).

This concept is based on the following facts: 1) There are special pharmacological features of the inhibitory effects of adrenaline and allied substances. For example, denervation and cocaine increase only slightly the inhibitory actions of adrenaline, but potentiate markedly the excitatory actions. Ergot alkaloids, yohimbine and many synthetic adrenergic substances inhibit specifically the excitatory actions of adrenaline, in the same series of aminomethylbenzodioxane derivatives, some compounds (1081 F or methoxy-2-iodo-5-phenoxyethyl-diethyl-

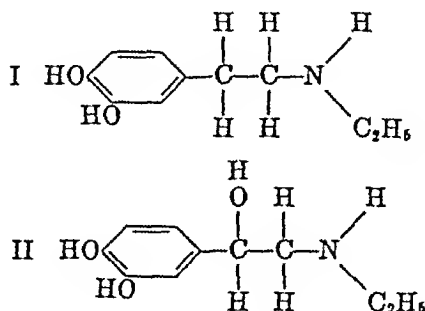
<sup>10</sup> A regrettable confusion of terms exists in the literature, one finds "cholinergic" or "adrenergic" instead of choline-like or adrenaline-like. According to Dale (169), who coined these words, only a nerve can be cholinergic or adrenergic.

amine, for example) abolish the inhibitory effects of adrenaline, but do not inhibit the excitatory actions (58, 176)

2) In the series of the catechol derivatives, there is a subtle correlation between chemical structure and intensity of inhibitory action Barger and Dale (183) had demonstrated that the presence of a single methyl group on the nitrogen is a



determinant factor for true inhibitory activity (for example, on the non-pregnant cat's uterus, the pure test for inhibition<sup>11</sup>), noradrenaline (without a methyl group) and methedrine (with two  $-CH_3$  radicals) have little inhibitory power. Recent investigations have confirmed this law, but the importance of hydroxyl group on the  $\beta$ -carbon of the side-chain has also been stressed (185, 186). The best demonstration is the following: N-ethoxytyramine (I) is about twenty times less active than adrenaline on the basis of tests for excitation (nictitating membrane, blood pressure) and has almost no inhibitory actions (non-pregnant cat's uterus, blood pressure after 933 F), on the contrary, N-ethylarterenol (II), which also is much less active than adrenaline in the test for excitation, has *qualitatively* and *quantitatively* the same powerful inhibitory effects as adrenaline (186).



This must be correlated with differences in their chemical behavior: arterenol (very weak inhibitor) does not oxidize to an "arterenolochrome" corresponding

<sup>11</sup> It might be useful to call attention to the fact that the isolated mammalian or birds' intestine is not a pure test for inhibition because adrenaline acts on the neurones which regulate the automatism of this smooth muscle (184). A great deal of the discrepancies found in the literature as regards the inhibitory action of sympathomimetic amines is due to the use of the intestine as the test for inhibition. Cushny, Dale and Cannon have always chosen for this purpose the faithful uterus of the non-pregnant cat.

the substrate for melanin formation in the iris. In the young rabbit, sympathetic denervation of the eye results in the lack of pigmentation, thus, one can reproduce experimentally the heterochromia known in human patients to be associated with congenital absence of sympathetic innervation. If adrenaline solutions are instilled in the conjunctival sac, one succeeds not only in producing normal pigmentation but sometimes hyperpigmentation. Thus it is the adrenaline (or adrenaline-like substance) constantly liberated in the iris by the adrenergic nerves which is the substrate for the phenolase of that organ. To be sure, only a negligible fraction of adrenaline is metabolized to melanin, albino rabbits exhibit the same response and sensitivity to adrenaline as do black rabbits (168).

*F Oxidized derivatives, beyond adrenochrome, obtained in vitro* With biological tests, one can obtain the most contradictory results, because solutions of more or less "oxidized adrenaline" are mixtures of many substances in variable proportions. The best demonstration of this basic fact is the following. If one oxidizes a  $1 \times 10^{-4}$  solution of adrenaline at room temperature and neutral pH with a catecholoxidase, one obtains precipitate of melanin at a time when the red, clear solution still gives the physiological effects of adrenaline. Thus, in this solution all the intermediates are present together, with some of the unaltered substrate and the end-product. Under well-defined conditions, weaker solutions oxidize more uniformly.

Some authors obtained acetylcholine-like effects<sup>10</sup> with oxidized derivatives of adrenochrome (170, 171), but if one adds cysteine these effects are inhibited. Fresh solutions of adrenochrome and cysteine should have an adrenaline-like action. Gijon (172) states that adrenaline solutions which are autooxidizing *in vitro* have a powerful contracting effect on isolated uteri, and that this effect is inhibited by adrenaline.

The pertinent literature is very confusing and it may be hoped that Harley's recent contribution will clarify the subject. Chemically speaking, it is possible that the secondary alcohol group is oxidized to an "oxoadrenochrome" which has not been isolated (173), it has been known for a long time that *l*-adrenaline oxidizing at an alkaline pH loses its optical activity (165) which adrenochrome still keeps (107). Melanin should be a polymer of this oxoadrenochrome (174).

A series of investigations by Bacq and Heirman has linked the inhibitory action of adrenaline with the production of a powerful inhibitory substance beyond adrenochrome (58, 177, 178, 179, 180, 181, 182).

This concept is based on the following facts: 1) There are special pharmacological features of the inhibitory effects of adrenaline and allied substances. For example, denervation and cocaine increase only slightly the inhibitory actions of adrenaline, but potentiate markedly the excitatory actions. Ergot alkaloids, yohimbine and many synthetic adrenolytic substances inhibit specifically the excitatory actions of adrenaline, in the same series of aminomethylbenzodioxane derivatives, some compounds (1081 F or methoxy-2-iodo-5-phenoxyethyl-diethyl-

<sup>10</sup> A regrettable confusion of terms exists in the literature, one finds "cholinergic" or "adrenergic" instead of choline-like or adrenaline like. According to Dale (169), who coined these words, only a nerve can be cholinergic or adrenergic.

## ADDENDUM\*

The presence of arterenol (noradrenaline) in some adrenal extracts (206, 208, 209), in adrenal medullary tumors (207) and in the secretion of the adrenals stimulated by the splanchnics (206) seems well established. The suggestion of Bacq and Fischer (10) that the tissues synthesize a mixture in variable proportions of adrenaline and noradrenaline is discussed by Goldenberg *et al* (211). U S von Euler and his pupils have actively continued their work on adrenaline and noradrenaline in tissue extracts (213 to 217). A method is given for purification and titration of noradrenaline in presence of adrenaline (213), a paper on the same subject by Gaddum, Peart and Vogt is in press in the *Journ Physiol*.

Further data have been published on adrenochrome, its derivatives and adrenaline ascorbate (212). B Kisch has summarized the pre-war work on the catalytic effects of oxidized adrenaline (205). Herve and Lecomte have shown that the semicarbazone of adrenochrome (*adrenozyl* Labaz) inhibits in the mouse the cutaneous purpura which follows a heavy dose of X-ray (218).

Fischer (219) has isolated, in confirmation of Harley-Mason's statement, a crystalline, yellow, highly fluorescent substance (M P 235° C) from adrenochrome in alkaline medium. This substance (formula VI, p 15) seems to be responsible for the fluorescence of adrenaline in alkaline solution. Many experiments of Utevsky have been confirmed. The relation between fluorescence and inhibitory action of catecholamines has been strengthened (220, 221).

## REFERENCES

1. BERNHEIM, F. The interaction of drugs and cell catalysts, Burgess Publishing Co., Minneapolis (1942)
2. BETER, K. H. Sympathomimeticamines: the relation of structure to their action and inactivation *Physiol Rev* 26 169 1946
3. BACQ, Z. M. Métabolisme et synthèse de l'adrénaline, *Bull Acad Méd. Suisse*, 4 127, 1948.
4. MALAFAYA BAPTISTA, A. Inactivação da adrenalina no organismo. Thesis, Lisbon, 1938.
5. HARTUNG W. H. Annual review of Biochemistry 15 523, 1946
6. ELLIOTT T. R. *Journ Physiol* 32 401, 1905
7. EULER, U. S. von. *Journ Physiol* 105 38, 1946
8. EULER, U. S. von. *Acta Physiol Scand*, 11 165, 1946 and 12 73 1946
9. SCHOTTERLÖW, C. G. *Acta Physiol Scand*, 15 47, 1948
10. EULER, U. S. von. *Science*, 107 422, 1948.
11. BACQ, Z. M., AND FISCHER, P. *Arch. Internat. Physiol* 55 73, 1947
12. BACQ, Z. M. *Science*, 108 135, 1948
13. GADDUM, J. H., AND GOODWIN, L. G. *Journ. Physiol.*, 105 357, 1947
14. TAYNTER, M. L., TULLAR, B. F., AND LUDVIG, F. P. *Science*, 107: 89, 1948
15. VERLEY, W. *Arch Internat. Physiol*, 55 409 1948
16. CANNON, W. B., AND ROSENBLUTH, A. *Amer Journ Physiol* 104 537 1933
17. CANNON, W. B., AND BACQ, Z. M. *Amer Journ. Physiol.*, 96 392, 1931
18. BACQ, Z. M. *Biol Rev* 22 73 1947
19. BAYER G., AND WENSE, TH. *Arch exper Path. Pharmacol.*, 183 114 1937
20. TOSCANO, RITO J. AND MALAFAYA, BAPTISTA A. *Compt rend Soc Biol*, 120: 545, 1935.
21. WENSE, TH. *Arch exper Path. Pharmacol.*, 191 358, 1939
22. BACQ, Z. M. *Arch Internat. Physiol*, 46 125 1938.
23. BACQ, Z. M. ET FREDERICQ H. *Arch Internat. Physiol*, 40 454, 1935
24. BACQ Z. M. LECOMTE, J. AND FISCHER P. *Arch Internat. Physiol* 1949 in press
25. BACQ, Z. M. *Arch Internat. Physiol*, 44 15 1936
26. BAIN, V. A., CAUNT W. E., AND SUFFOLK, S. F. *Journ Physiol*, 91 233, 1937
27. VERLEY W. *Arch Internat. Physiol* 56: 1 1948
28. BACQ Z. M. FISCHER, P. AND LECOMTE, J. *Arch Internat. Physiol*, 56 25, 1948
29. LOEWI O. *Pflüger's Arch*, 237 604 1936.

\* Added in galley



to adrenochrome (187) and does not give the marked fluorescence of adrenaline after addition of NaOH (117). The same phenomenon occurs with the N-ethyl derivatives: substance II gives the Gaddum and Schild fluorescence test, like adrenaline, substance I behaves like arterenol. It is clear from the investigations of Cohen (173, 174) and Harley-Mason (114) that the secondary alcohol group does play an important rôle in the oxidation and reduction of adrenochrome.

3) When an isolated amphibian heart is impregnated with a phenolase preparation, the excitatory reaction to adrenaline is changed to inhibition (177) and sympathetic nerve excitation decreases the rate and weakens the strength of the heart beat (188, 189), these reversed effects are progressively changed to normal when the phenolase is washed out.

4) Dilute solutions of adrenaline at neutral pH when oxidized *in vitro* by a phenolase, irregularly exhibit, after an initial phase of complete inactivation, a very powerful inhibitory action on the heart, blood vessels (179) and various smooth muscles (182, 190, 191). This inhibitory substance, called "adrenoxine", could not be isolated because it decomposes on concentration and is not produced from strong solutions of adrenaline. Many physiologists failed to reproduce Bacq and Heirman's experiments (172, 192, 164), but some have confirmed them at least partially (192, 193, 194, 195, 196). Older observations of Schweitzer, with the omega substance (198), are quite consistent with the results of Heirman. Marquardt (197) quite frequently observed the depressor action of oxidized adrenaline and believes that it is a general property of phenols and indole. Starkenstein (199) has described a curious "Sauerstoffphänomen", the inhibitory action of adrenaline on isolated intestine is abolished by lack of oxygen.

In 1940, Bacq and Heirman (58) summarized the facts in favor of and the objections to the adrenoxine theory.

**VIII OTHER POSSIBLE MECHANISMS OF INACTIVATION** Aldehydes (formaldehyde, acetaldehyde or methylglyoxal) which inactivate adrenaline *in vitro* (18, 19, 20) do not seem, for many reasons, to take part in its inactivation *in vivo* (21), but this opinion has been contested (4). Nothing indicates that the ring of adrenaline cannot be broken just as that of tyramine seems to be broken by heart and skeletal muscle tissue (104).

#### SUMMARY

The statement formulated in 1905 by Elliott (5) that "adrenalin disappears in the tissues which it excites" is unquestionable. Review of the literature shows 1) that adrenaline may be excreted unchanged in small amounts by the kidneys and stored in the tissues and red blood cells, 2) that its deamination by amine-oxidase in the body is unlikely, 3) that an important fraction is sulfoconjugated, 4) that another important fraction is simultaneously oxidized to indole substance, 5) that adrenochrome and its derivatives have important biochemical and physiological properties, entirely different from those of adrenaline, and deserve further study.

## ADDENDUM\*

The presence of arterenol (noradrenaline) in some adrenal extracts (206, 208, 209), in adrenal medullary tumors (207) and in the secretion of the adrenals stimulated by the splanchnics (206) seems well established. The suggestion of Bacq and Fischer (10) that the tissues synthesize a mixture in variable proportions of adrenaline and noradrenaline is discussed by Goldenberg *et al* (211). U S von Euler and his pupils have actively continued their work on adrenaline and noradrenaline in tissue extracts (213 to 217). A method is given for purification and titration of noradrenaline in presence of adrenaline (213), a paper on the same subject by Gaddum, Peart and Vogt is in press in the *Journ Physiol*.

Further data have been published on adrenochrome, its derivatives and adrenaline ascorbate (212). B Kisch has summarized the pre-war work on the catalytic effects of oxidized adrenaline (205). Herve and Lecomte have shown that the semicarbazone of adrenochrome (*adrenoryl* Labaz) inhibits in the mouse the cutaneous purpura which follows a heavy dose of X-ray (218).

Fischer (219) has isolated, in confirmation of Harley-Mason's statement, a crystalline, yellow, highly fluorescent substance (M P 235° C) from adrenochrome in alkaline medium. This substance (formula VI, p 15) seems to be responsible for the fluorescence of adrenaline in alkaline solution. Many experiments of Utevsky have been confirmed. The relation between fluorescence and inhibitory action of catecholamines has been strengthened (220, 221).

## REFERENCES

1. BERNHEIM, F. The interaction of drugs and cell catalysts. Burgess Publishing Co., Minneapolis (1942)
2. BEYER, K. H. Sympathomimetic amines: the relation of structure to their action and inactivation, *Physiol Rev* 26 169 1946
3. BACQ, Z. M. Métabolisme et synthèse de l'adrénaline, *Bull Acad Méd Suisse* 4 127, 1948
4. MALAFAYA BAPTISTA, A. Inactivação da adrenalina no organismo. Thesis. Lisbon 1938.
5. HARTUNG W. H. Annual review of Biochemistry 15 593 1946.
6. ELLIOTT T. R. *Journ Physiol* 32 401, 1905
7. EULER, U. S. *Journ Physiol*, 105 38, 1946.
8. EULER, U. S. *Acta Physiol Scand.*, 11 163, 1945 and 12 73, 1946
9. SCHMITTERLOW, C. G. *Acta Physiol Scand.* 15: 47 1948
10. EULER, U. S. *Science*, 107: 422 1948
11. BACQ Z. M., AND FISCHER, P. *Arch. Internat. Physiol.* 55 73, 1947
12. BACQ, Z. M. *Science*, 103 135 1948
13. GADDUM, J. H., AND GOODWIN, L. G. *Journ Physiol.*, 105 357, 1947
14. TAINTER, M. L., TULLAR, B. F. AND LUDUENA, F. F. *Science* 107 39, 1948
15. VERLY, W. *Arch. Internat. Physiol.*, 55 409 1948
16. CANNON W. B., AND ROSENBLUTH, A. *Amer Journ Physiol*, 104 537 1933
17. CANNON W. B. AND BACQ Z. M. *Amer Journ Physiol*, 96 392, 1931
18. BACQ Z. M. *Biol. Rev.* 22 73 1947
19. BAYER G., AND WENZEL, TH. *Arch exper Path. Pharm.*, 168 114, 1937
20. TOSCANO, RICO J., AND MALAFAYA, BAPTISTA A. *Compt rend. Soc Biol* 120-545 1935
21. WENZEL, TH. *Arch exper Path. Pharmacol.*, 191 355, 1939
22. BACQ Z. M. *Arch Internat. Physiol* 46 125 1938
23. BACQ Z. M. ET FREDERICQ H. *Arch Internat. Physiol*, 40 454 1935
24. BACQ, Z. M. LECOMTE, J., AND FISCHER P. *Arch Internat. Physiol* 1949 in press
25. BACQ Z. M. *Arch Internat. Physiol* 44 15 1938
26. BAILEY, A., GAUNT, W. E. AND SUFFOLK, S. F. *Journ. Physiol*, 91 233, 1937
27. VERLY, W. *Arch. Internat. Physiol.*, 56 1 1948
28. BACQ Z. M., FISCHER, P. AND LECOMTE, J. *Arch. Internat. Physiol*, 56 25 1948
29. LOWY, O. *Pflüger's Arch* 237 504 1938.

\* Added in galley

- 29 CANNON, W B, AND LISSAK K. *Amer Journ Physiol*, 125 765, 1939
- 30 SHAW, F H *Biochem Journ*, 32 19 1938
- 31 RAAB, W *Biochem Journ*, 37 470, 1943
- 32 RAAB, W, AND HUMPHREYS, R J *Amer Journ Physiol*, 148 460, 1947
- 33 BACQ, Z M *Arch Internat. Physiol*, 36 167, 1933
- 34 RAAB, W, AND MAES, J P *Amer Journ Physiol*, 148 470, 1947
- 35 ENOELHART, E *Pflüger's Arch*, 227 220, 1931
- 36 CHANG H C, AND GADDUM, J H *Journ Physiol* 79 255, 1933
- 37 MACINTOSH, F C *Arch Internat Physiol*, 47 321, 1938
- 38 RAAB, W AND HUMPHREYS, R J *Journ Pharm exper Ther*, 88 288 1946
- 39 BÜLBRING E BJRN, J H, AND DE ELIO F J *Journ Physiol*, 107 222, 1948
- 40 WEST, G B *Journ Physiol*, 106 418, 1947
- 41 VERLY, W *Arch Internat Physiol*, 55 397, 1948
- 42 JAMES W O *Nature* 161 851, 1948
- 43 KALAJA, L, AND SAVOLAINEN H *Nord Med*, 12 3502, 1941
- 44 LEHMAN, G AND MICHAELIS, H F *Arbeitsphysiol*, 12: 52 and 265 1942
- 45 JØRGENSEN, K S *Acta Pharmacol et Toxicol (Copenhagen)*, 1: 225, 1945
- 46 PORAT B, VON, *Acta Med scandin*, 123 317, 1946
- 47 RAAB, W *Amer Heart Journ*, 33 707, 1947
- 48 TRENDLENBURG P *Die Hormone*, Springer, Berlin, 1929
- 49 BACQ, Z M *Arch Internat Physiol*, 44 15, 1938
- 50 WEST, G B *Journ Physiol*, 106 426, 1947
- 51 MACKENZIE, C G, CHANDLER J P, KELLER, E B, RACHELE, J R, CROSS, N, MELVILLE, D B, AND DU VIGNEAUD V *Journ Biol Chem*, 169: 757, 1947
- 52 KRÜGER M, AND SCHMIDT J *Arch exp Path Pharm*, 45 259, 1901
- 53 VIGNEAUD, V DU, CHANDLER J P, SIMMONDS, S, MAYER, A W, AND COHN, M *Journ. Biol Chem*, 164: 603, 1946
- 54 BLASCHKO H, RICHTER, D, AND SCHLOSSMANN, H *Journ Physiol*, 90: 1, 1937
- 55 RICHTER D *Bloch Journ*, 31 2022, 1937
- 56 HOLTZ P, AND HEISE, R *Arch exper Path Pharm*, 190: 178 1938
- 57 HOLTZ, P, RINGOLD, A, AND CHEDNER, K *Zeitsch Physiol Chem*, 261: 278, 1939
- 58 BACQ, Z M AND HEUBMAN, P *Arch Internat Physiol*, 50 163 1940
- 59 BLASCHKO, H, RICHTER, D, AND SCHLOSSMANN, H *Bioch Journ*, 31: 2187, 1937
- 60 ORZESCHOWSKI, G *Arch exper Path Pharm*, 198: 27, 1941
- 61 HARE, M L C *Bloch Journ* 22 968 1928
- 62 PUGH, C E M, AND QUASTEL J H *Bloch Journ*, 31: 286 and 2038, 1937
- 63 EWING, A J, AND LAIDLAW, P P *Journ Physiol* 41: 78 1910
- 64 GUGGENHEIM, M, AND LÖFFLER, W *Bloch Zeitsch*, 72 325, 1915
- 65 RICHTER, D *Bloch Journ*, 32: 1763, 1938
- 66 GADDUM J H, AND KWATKOWSKI, H *Journ Physiol*, 94 87 1938 and 96: 385, 1939
- 67 RICHTER, D, AND TINGEY, A H *Journ Physiol*, 97: 285 1939
- 68 SCHAPIRA, G *Compt rend Soc Biol*, 139 36, 1943
- 69 KOHN, H I *Biochem Journ*, 31 1693, 1937
- 70 MANKOWITZ, J, AND MANN, F C *Amer Journ. Physiol.*, 89: 176, 1929
- 71 BACQ Z M *Arch Internat Physiol*, 45: 1, 1937
- 72 BACQ, Z M *Arch Internat Physiol*, 44 112, 1936
- 73 PETRILLO, L M, AND BACQ Z M *Compt rend Soc. Biol*, 141: 839, 1947
- 74 BACQ Z M *Arch Internat Pharm Thé*, 55: 190, 1937
- 75 TRIFOD J *Journ Physiol*, 97 289, 1940
- 76 MACGREGOR, D F *Journ Pharmacol exper Ther*, 66 393, 1939
- 77 PHILPOT F J *Journ Physiol*, 97: 301, 1940
- 78 BACQ, Z M, AND LEFEBVRE F *Arch Internat. Pharm Thé*, 49: 363, 1935
- 79 WEINSTEIN S S AND MANNING R I *Science*, 86 19, 1937
- 80 RICHTER D *Journ Physiol*, 98 361 1940
- 81 FLOSKIN, M, AND BACQ Z M *Arch Internat Physiol* 53: 247 1943
- 82 WILLIAMS R T *Detoxication mechanisms* Chapman and Hall London 1947
- 83 THIERFELDER, H AND SHERWIN, C P *Zeitsch physiol Chem*, 94 1, 1913
- 84 PREUBSE C *Zeitsch physiol Chem* 2 324 1878
- 85 BACQ Z M *Annales Physiol* 10 467 1934
- 86 RICHTER D, AND MACINTOSH, F C *Amer Journ Physiol*, 135 1 1941
- 87 BEYER, K H AND SHAPIRO, S H *Amer Journ Physiol*, 144 321, 1945
- 88 DRICHMANN, W B *Proc Soc exper Biol Med* 54: 335 1943
- 89 ARNOLD R I AND DE MEIG R H *Rev Soc Argent Biol*, 17 570, 1941
- 90 MARENZI A D *Compt rend Soc Biol*, 107: 737 and 743, 1931
- 91 BARAC G *Arch Internat Pharm Thé* 56 13, 1937
- 92 LOEFER M LOEFER J LEMIRE J, COTTET A, AND PARROT J *Compt rend Soc Biol* 123: 1030 1938
- 93 BACQ Z M LECOUTE J, AND FISCHER, P *Arch Internat Physiol*, 1949 in press

- 94 HOLTZ, P., CREDNER, K., AND KRONENBERG, G. *Arch exper Path Pharmacol*, 204: 228, 1947
- 95 TORDA, C. *Journ. Pharmacol exper Ther*, 77: 123, 1943
- 96 TORDA, C. *Journ. Pharmacol exper Ther*, 77: 274, 1943
- 97 BACQ, Z. M. *Arch Internat Physiol*, 42: 340, 1936
- 98 ANDERHALDEN, E., AND GELLHORN, E. *Pflüger's Archiv*, 203: 42, 1924
- 99 EERFORS, H. *Compt. rend Soc Biol*, 93: 1162, 1925
- 100 DE CARO, L. *Zeitsch physiol. Chem*, 219: 257, 1933
- 101 MACKAY SAWYER M. E., AND BROWN, M. G. *Amer Journ physiol*, 110: 620, 1933
- 102 DE VISECHER, M. *La régulation hormonale du métabolisme et la vitamine A*, Masson, Paris, 1946
- 103 WELACH, A. D. *Amer Journ Physiol*, 103: 360, 1934
- 104 BERNHEIM F., AND BERNHEIM, M. L. C. *Journ. Biol Chem*, 153: 369, 1944.
- 105 GUOONHEIM, M. *Die biogenen Amine*, Karger, Basel, 1940
- 106 RAPEK, H. S. *Biochem Journ*, 21: 89, 1927
- 107 GREEN, D. E., AND RICHTER, D. *Biochem. Journ*, 31: 595, 1937
- 108 DEROUAUX, G. *Arch Internat Pharmac Thé*, 69: 203, 1943
- 109 POLOKOVSKI, M. *Biochimie Médicale*, Masson, Paris, 1948.
- 110 BALL, E. G., AND CHEN, T. T. *Journ Biol Chem*, 102: 691, 1933
- 111 MARQUARDT, P. *Enzymologia*, 12: 165, 1947
- 112 OTEVSKY, A. M. *Nouvelles biologiques soviétiques*, 18: 145, 1944.
- 113 GUON, J. R. *Trabajos Inst Cajal*, 3: 73, 1945
- 114 HARLEY MASON, J. *Experientia*, 4: 307, 1948.
- 115 VEER, W. L. C. *Recueil trav chim. Pays Bas* 61: 638, 1942
- 116 BRACONIER, F., LE BIRAN, H. AND BEAUDET, C. *Arch Internat Pharm. Thé*, 69: 181, 1943
- 117 GADDUM, J. H., AND SCHULZ, H. *Journ Physiol*, 80: 9, 1934.
- 118 PARROT, J. L. *Compt. rend Soc Biol*, 142: 631, 1948.
- 119 BACQ, Z. M., LECOMTE, J., AND BEAUDET, C. *Unpublished observations*, 1947-1948.
- 120 KIRCH, B., AND COLL. Numerous papers in *Bioch. Zeitsch*, 220 to 271: 1930 to 1934
- 121 CAHEN, A. *Compt. rend. Soc. Biol.*, 139: 22, 1945
- 122 FRIEDHEIM, E. A. H. *Compt. rend Soc Phys. Hist. Nat. Genève*, 50: 20, 1933, *Bioch. Zeitsch*, 259: 257, 1933, *Schweiz. Med. Wochensch.*, 65: 256, 1935
- 123 EVANS, W. C., AND RAPEK, H. S. *Biochem Journ*, 31: 2162, 1937
- 124 BLIX, G. *Skand Archiv Physiol*, 56: 131, 1929
- 125 WATZER, J. *Bull. Soc. Chim. Biol*, 23: 341, 1945
- 126 DEROUAUX, G. *Arch. Internat. Pharmac Thé*, 66: 202, 1941
- 127 DEROUAUX, G. *Arch. Internat. Pharmac Thé*, 69: 142, 1943
- 128 ROSKAM, J., DEROUAUX, G., MEYS, L., AND SWALUÉ, L. *Arch. Internat. Pharmac Thé*, 74: 162, 1947
- 129 ROSKAM, J., AND DEROUAUX, G. *Arch. Internat. Pharmac Thé*, 69: 12, 1943
- 130 BACQ, Z. M. *Presse Médicale*, 175: March 15, 1947
- 131 BACQ, Z. M. *Compt. rend. Soc. Biol.*, 141: 536, 1947
- 132 PARROT, J. L., AND LAVOILLAT, J. *Compt. rend. Acad. Sciences*, 218: 211, 1944.
- 133 PREYNOT, H., COTTEBAU, H., AND PARROT, J. L. *Compt. rend. Soc. Biol.*, 141: 1043, 1947
- 134 PARROT, J. L., AND COTTEBAU, H. *Compt. rend. Soc. Biol.*, 139: 902, 1945
- 135 JAVILLIER, M., AND LAVOILLAT, J. *Helvetica Chimica Acta*, 29: 1283, 1946
- 136 ROSKAM, J., AND DEROUAUX, G. *Bull. Acad. Roy. Méd. Belg*, 5th series, 10: 83, 1943
- 137 WATZER, J. *Bull. Soc. Chim. Biol*, 23: 345, 1945
- 138 MEYERHOF, O., AND RANDALL, L. O. *Archiv Biochem.*, 17: 171, 1948.
- 139 LECOMTE, J., AND FISCHER, P. *Arch Internat Physiol*, 56: 35, 1948.
- 140 GOTTART, M. *Compt. rend. Soc. Biol.*, in press, 1949
- 141 LETTÉ, H. *Chemiker Zeitung*, 67: 52, 1943
- 142 LETTÉ, H., AND ALBRECHT, M. *Zeitsch. Physiol. Chem.*, 271: 200, 1941
- 143 BACQ, Z. M. *Experientia*, 2: 349 and 385, 1946
- 144 SOLOWAY, S., AND OSTER, K. A. *Proc. Soc. exper Biol Med.*, 50: 108, 1942
- 145 OSTER, K. A., AND SOBOTKA, H. *Journ. Pharmacol. exp. Ther.*, 78: 100, 1943
- 146 QUIVY, D., AND CLONT, L. *Compt. rend. Soc. Biol.*, 141: 1173, 1947
- 147 HALSE, TH., AND MARQUARDT, P. *Enzymologia*, 12: 245, 1948
- 148 MARTIN, F. N. *Proc. Soc. exper Biol Med*, 59: 1, 1945
- 149 SNYDER, F. H., LEVY, L., AND OBERST, F. W. *Journ. Amer. Pharm. Assoc*, 36: 253, 1947
- 150 WATZER, J. *Bull. Soc. Chim. Biol*, 29: 237, 1947
- 151 CALDETRO-BARCIA, R. *Arch. Soc. Biol. Montevideo*, 13: 183, 1945.
- 152 NEUBERGER, A. *Annual reports of Chem. Soc.*, 41: 237, 1944
- 153 BLASCHKO, H. *Journ. Physiol.*, 101: 337, 1942
- 154 POLOKOVSKI, M., GONNARD, P., AND PELOU, A. *Bull. Soc. Chim. Biol.*, 26: 449, 1944
- 155 VIKET, A. *Bull. Soc. Chim. Biol.*, 22: 532, 1940
- 156 HOLTZ, P., CREDNER, K., AND LUEDTKE, K. *Archiv, exper. Path. Pharmacol.*, 191: 87, 1938
- 157 GURIN, S., AND DELLUYA, A. M. *Journ. Biol. Chem.*, 170: 545, 1947
- 158 LECOMTE, J., AND FISCHER, P. *Arch. Internat. Physiol.*, 1949: in press
- 159 NIHOUL, E., LECOMTE, J., AND FISCHER, P. *Compt. rend. Soc. Biol.*, 1949: in press
- 160 BHAGVAT, K., AND RICHTER, D. *Bioch. Journ.*, 32: 1397, 1938.

- 161 DUCHATEAU BOSSON, AND FLORKIN, M Compt rend Soc Biol., 132 47, 1939
- 162 KEILIN, D, AND HARTREY, E F Proc Roy Soc B, 119: 114 1936
- 163 KEILIN, D, AND HARTREE, E F Proc Roy Soc B, 125 171, 1938
- 164 BLASCHKO, H, AND SCHLOSSMANN, H Journ Physiol, 98 130, 1940
- 165 SCHÜTZ, F Bloch Zeitsch, 265 282, 1933
- 166 BENNETT G A, AND HAUSBERGER, F X Archiv exper Path Pharmac, 188: 40, 1938
- 167 CLARK, A J, AND RAVENTOS, J Quart Journ exper Physiol, 29: 159, 1939
- 168 HEIRMAN, P Compt rend Soc Biol, 126 1267, 1937
- 169 DALE, H H Journ Physiol, 80 10 P, 1933
- 170 MINE, B, AND PLOTKA, C Compt rend Acad Sciences, 225: 697, 1947
- 171 PLOTKA, C, AND JEQUER, R Compt rend Soc Biol, 141 1190 and 1191, 1947
- 172 GIJON, J R Trab Instit Cajal, 3 193, 1945
- 173 COHEN, G N Bull Soc Chim Biol, 28 104, 1945
- 174 COHEN, G N Bull Soc Chim Biol, 28: 107, 1946
- 175 FRIEDMANN, E, MARRIAN, D H, AND SIMON-REZUS, I Brit Journ. Pharmacol. Chemoth, 3 263, 1948.
- 176 BOVET, D, AND SIMON, A Arch Internat Pharmac Ther, 55: 223, 1937
- 177 HEIRMAN, P Arch Internat Physiol, 46: 404, 1938
- 178 HEIRMAN, P Arch Internat Physiol, 49: 449, 1939
- 179 HEIRMAN, P, AND BACQ, Z M Arch Internat Physiol, 50 100, 1940
- 180 HEIRMAN, P Arch. Internat Physiol, 50 115, 1940
- 181 BACQ, Z M, AND HEIRMAN, P Arch Internat Physiol, 50 129, 1940
- 182 BACQ, Z M Arch Internat Physiol, 50: 141, 1940
- 183 BARGER, G, AND DALE, H H Journ Physiol, 41: 19, 1910
- 184 NOLP, P Mélanges J Demoor, Masson, Paris, 1937, p 278.
- 185 MARSH, D F, PELLETIER, M H, AND ROSS, C A Journ. Pharmac exper Ther, 92 108, 1948.
- 186 BACQ, Z M, LECOMTE, J, AND FISCHER, P Arch Internat Physiol, in press, 1949
- 187 BEAUDET, C Personal communication, 1948
- 188 BACQ, Z M Arch Internat Physiol, 46 417, 1938
- 189 CALDETEO-BANCIA, R Arch Soc Biol Montevideo, 13: 67, 1946
- 190 GOFFART, M Compt rend. Soc Biol, 130 1372, 1939
- 191 HEIRMAN, P, AND GOFFART, M Compt rend Soc Biol., 132 84, 1939
- 192 LISSAK, K. Science, 67 371, 1938
- 193 SANDELS, E Arch exper Path Pharm, 193 572, 1939
- 194 VEIL, C Compt rend Soc Biol, 132 800 1939
- 195 UNGAR, G, AND PARROT, J L Compt rend Soc Biol, 131: 62 and 1165, 1939
- 196 PARROT, J L, AND VERILLAC, F Compt. rend Soc Biol, 136 323, 1942
- 197 MARQUARDT, P Enzymologia, 12 166, 1947
- 198 SCHWEITZER, A Pflüger's Archiv, 228 263, 1931
- 199 STARKENTHEIN, E Arch Internat Pharm Thé, 65 423, 1941
- 200 SEITZ, W Zeitschr exper Med, 105 559, 1939
- 201 DOGSON, K S, GARTON, G A, AND WILLIAMS, T R Bloch Journ, 41: L, 1947
- 202 LIPSCHITZ, W L, AND BUDING, E Journ Biol Chem., 129: 333, 1939
- 203 MARQUARDT, P, AND OETTEL, H Arch Internat Pharm Thé, 77: 180, 1948.
- 204 DUSTIN, P Compt. rend Soc Biol, 1949, in press.
- 205 KIRSCH, B Exper Med Surg 5 70, 1947
- 206 BÜLBRING E, AND BURN J H Nature, 163: 363, 1949
- 207 HOLTON P Nature, 163 217 1949
- 208 HOLTZ, P, AND SCHÜMMANN, H J Naturwiss 35 159, 1948
- 209 HOLTZ, P, SCHÜMMANN, H J LANGENBECK, W AND LE BLANC, H Naturwiss, 35: 191, 1948
- 210 HOLTZ, P, AND SCHÜMMANN, H J Arch exper Path Pharm 1949, in press
- 211 GOLDENBERG M, PINES, K L, BALDWIN, E DE F, GREENE, D G., AND ROH, C E Amer Journ Med, 5 792, 1948
- 212 BEAUVILLAIN, A, AND SARRADIN, J Bull Soc Chim Biol, 30 472 and 478 1948
- 213 EULER, U S VON Arch Internat Pharm Thé, 77 477, 1948
- 214 EULER, U S VON Schweiz Med Wochschr 78 777, 1948
- 215 EULER, U S VON Nature, 162 570, 1948
- 216 EULER, U S VON Acta Physiol Scand, 16 63, 1948
- 217 SCHMITERLÖW, C G Acta Physiol Scand 16 suppl 58 1948
- 218 HERVE, A AND LECOMTE J Arch Intern Pharm Thé 1949 in press
- 219 FISCHER P Unpublished observations
- 220 BACQ, Z M, AND LECOMTE, J Arch Intern Pharm Thé 78 369, 1949
- 221 BACQ, Z M, AND FISCHER, P Compt rend Soc Biol, 1949 in press

# THE PHARMACOLOGY OF ADRENERGIC BLOCKADE

MARK NICKERSON

*Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah*

Among agents selectively blocking the effects of various portions of the nervous system, drugs inhibiting responses to the sympatho-adrenal division have always been the least satisfactory. Over forty years ago, Dale (86) clearly defined the action of ergot in blocking and "reversing" many responses to circulating epinephrine and sympathetic nerve stimulation, but subsequent progress in the field has been slow. The use of adrenergic blocking agents in research and therapy has been seriously handicapped by the lack of specificity, the incompleteness of action and the high toxicity of available agents.

Agents which induce or block responses of sympathetically innervated effector cells may be conveniently grouped under the term "adrenergic" proposed by Dale (88). The term "adrenergic blocking agent" is employed in this review to designate compounds which specifically inhibit certain responses of effector cells to epinephrine, related amines and sympathetic nerve impulses. The action of adrenergic blocking agents is quite distinct from the action of substances which can prevent a sympatho-adrenal discharge by blocking nervous impulses at ganglia (tetraethylammonium ions), along peripheral neurons (local anesthetics) or within the central nervous system (barbiturates and other central nervous system depressants). The absurdity and complication involved in referring to barbiturates as adrenergic blocking agents or "sympatholytics" are obvious. It is equally confusing to refer, as some authors do, to the central nervous system effects of the ergot alkaloids as "sympatholytic."

The term "adrenergic blocking agent" is preferred to "sympatholytic" and "adrenolytic." Neither nerve ending, nor mediator nor effector cell is "lysed" by these agents (see also 90). The term "adrenolytic" has been employed more correctly by some authors (91) to refer to those factors responsible for the destruction or inactivation of epinephrine in tissue. In addition, any sharp distinction between "adrenolysis" and "sympatholysis" appears to be artificial. Although certain compounds (e.g., 933F) block the effects of circulating epinephrine considerably more effectively than they block responses to sympathetic nerve activity, this does not constitute a qualitative distinction. All adrenergic blocking agents appear to be more effective against responses to circulating sympathomimetic agents than against responses to sympathetic nerve activity, all degrees of effectiveness against these two stimuli may be found within the group, and even within a single series of compounds (47).

The present review is concerned largely with the major lines of progress in the field of adrenergic blockade during the past decade. No attempt has been made to include all publications relating to the subject during this period. Older work has been sighted only when necessary to help clarify recent contributions. Many details of the older work in this field are enumerated in the recent book of Bovet and Bovet-Nitti (40).

Extended consideration is given to the actions of the various agents other than the production of adrenergic blockade, in order to provide a basis upon which to evaluate experimental results. The literature on adrenergic blockade is replete with reports which merely record a single over-all effect of a blocking agent without any experimental analysis of the mechanism involved. Emphasis on the fact that most adrenergic blocking agents have multiple and complex pharmacological properties may serve to call attention to the inadequacy of much previous experimentation in this field.

### I $\beta$ -HALOALKYLAMINES

The  $\beta$ -haloalkylamines, of which Dibenamine (N,N-dibenzyl- $\beta$ -chloroethylamine) may be considered as the prototype, represent the most recently discovered series of adrenergic blocking agents. At the present time, the blockade produced by members of this group of compounds appears to be more complete and more specific than that produced by members of other series. Although N,N-dibenzyl- $\beta$ -chloroethylamine was characterized in the American literature by Eisleb in 1934 incidental to a patent description of certain synthetic intermediates (102), its pharmacology was first reported by Nickerson and Goodman (285) in 1945. Additional reports soon appeared describing more completely the pharmacology of Dibenamine and of certain congeners whose activity was suggested by an early report of the structure-activity relationship within the group (296). In general, only quantitative differences between the various active members of this series have been reported, consequently the properties of Dibenamine, the most thoroughly studied compound, may be considered representative except where different properties of other agents are specifically mentioned. Many pharmacological characteristics of members of this group are included in summaries which appeared during the past year (279, 287).

Adrenergically active  $\beta$ -haloalkylamines have a very low aqueous solubility, except in the presence of high acidity, and undergo quite rapid decomposition in neutral or alkaline aqueous solutions to form readily soluble alcohol derivatives (288). They may be prepared as stable stock solutions in acidified propylene glycol or alcohol (286). Decomposition in aqueous solution may account for one report (31) which failed to confirm many of the generally substantiated pharmacological observations on Dibenamine.

#### A *Adrenergic blocking action*

*1 Responses to injected epinephrine* The most prominent action of Dibenamine is a specific blockade of certain excitatory responses to epinephrine and sympathetic nerve activity (286), an observation which has been confirmed and extended by numerous investigators.

Careful injection of moderate doses of Dibenamine into anesthetized animals (286, 408) or normal recumbent humans (158, 183) causes little change in blood pressure. Larger doses induce a moderate, slow decrease in pressure. This has been noted particularly in unanesthetized dogs (411). The drug has been re-

ported to cause some rise in blood pressure in certain schizophrenic patients (258) Rapid injection may lead to a precipitous fall in blood pressure which is probably unrelated to adrenergic blockade

Pressor responses to exogenous and endogenous epinephrine and the direct pressor effect of splanchnic nerve stimulation are blocked and reversed in most species of animals The depressor response to splanchnic nerve stimulation in adrenalectomized animals administered Dibenamine is small (286) and similar to the effect seen after large doses of ergotamine (87) It is of interest that little or no reversal of the pressor response to epinephrine is observed in the rabbit, an animal in which adrenergic vasodilatation is apparently insignificant (65) Also, reversal is observed less regularly in the pithed cat (4) a preparation in which a high degree of vasodilatation exists prior to the administration of epinephrine Reversal is presumably due to an inhibition of adrenergic vasoconstriction with a consequent unmasking of adrenergic vasodilatation In chickens Dibenamine causes no reversal and even fails to inhibit the pressor action of epinephrine and most other sympathomimetic amines (397) A similar resistance of fowl to adrenergic blockade by ergot was noted many years ago (22, 86), and raises some interesting questions in comparative pharmacology The action of Dibenamine in other species of birds has not been studied

Minimal effective doses of the  $\beta$ -haloalkylamines may block the pressor effects of small but not large doses of injected epinephrine However, adequate blocking doses ordinarily produce a complete blockade, i.e., responses to all doses of epinephrine (as great as 10 mgm /kgm intravenously) are completely reversed (286) This high "effectiveness," as distinct from "potency," (see I, C-2), is evident by other tests of Dibenamine action such as inhibition of retraction of the cat's nictitating membrane in response to nerve stimulation, epinephrine and norepinephrine (292), prevention of cyclopropane-epinephrine cardiac arrhythmias (298), and inhibition of epinephrine vasoconstriction in frog extremities (155) Dibenamine blockade develops quite slowly even after intravenous administration (286, 373), this is probably due to the necessity for *in vivo* formation of active intermediates (see I, D-1)

The adrenergic blockade produced by members of the Dibenamine series is not significantly altered by anesthetic agents (286) and is not overcome by cocaine (295) Comparable results have been obtained with unanesthetized and pithed animals and those under barbiturate, urethane, ether and cyclopropane anesthesia This is in contrast to the well known inhibition of ergot blockade by barbiturates (see 66) However, heparin may significantly reduce the effectiveness of small doses of Dibenamine (295), presumably on the basis of a direct interaction similar to that occurring between the  $\beta$ -haloalkylamines and thio-sulfate (see I, D-1)

Dibenamine (286, 312) and several congeners (197, 240, 295) provide marked protection against the lethal effects of epinephrine Protection of this type in mice has been employed as an assay for adrenergic blocking activity (197, 240) Although the results are roughly parallel to those obtained by assay based on reversal of the pressor response to epinephrine in anesthetized cats (295), wide



quantitative discrepancies exist (see also I, D-3) Methacholine (240) and papaverine (313) have also been shown to provide significant protection against epinephrine toxicity in rodents, although they are not adrenergic blocking agents

The lack of specificity observed in protection against epinephrine toxicity is probably related to the mechanism of epinephrine-induced death in mice and rats. It has been shown that death from intraperitoneally administered epinephrine in rats is primarily due to respiratory failure and that the rise in systemic arterial pressure *per se* is an important etiological factor in the observed respiratory embarrassment (282). Systemic hypertension obviously does not play a comparable role in epinephrine toxicity in fowl. Although Dibenamine provides significant protection in fowl, it does not alter the pressor response to injected epinephrine (397).

The role of systemic arterial pressure in death due to epinephrine raises the possibility that any agent with a significant vasodepressor action could provide protection. Several non-specific agents providing no protection under certain experimental conditions (240) may afford protection when administered by selected routes at appropriate time intervals. Care must therefore be employed in interpreting the results of such experiments.

Accumulation of epinephrine of "epinephrine-like" substances in heart muscle was found not to be causally related to epinephrine toxicity in rats (282), although such a relationship has been previously stressed (311, 312). Inasmuch as most of the myocardial effects of epinephrine are unaltered by Dibenamine (see I, A-4), the lack of significance of myocardial accumulation of epinephrine might have been anticipated.

2 *Responses to sympatho-adrenal stimulation* The pressor effects of chemically-induced generalized sympatho-adrenal discharge are also inhibited by Dibenamine. Reversal of the nicotinic pressor action of acetylcholine and carbachol in atropinized animals has been regularly observed (286, 408), whereas the response to nicotine has been variously reported to be only inhibited (408) or to be completely reversed (217). Neither of these reports provides sufficient data to allow an adequate explanation of the divergent results. Failure to block completely the pressor response to nicotine may be due to a direct action of nicotine on peripheral vessels (155, 157, 246), which is non-adrenergic in nature and therefore not blocked by Dibenamine or ergotamine (246). An alternative explanation for the difference between the effects of Dibenamine on pressor responses to acetylcholine and nicotine is that sympathetic ganglion cells may differ in their pharmacological responses. It has been suggested that only ganglion cells subserving vasoconstrictor functions are readily stimulated by nicotine, whereas both those with vasoconstrictor and those with vasodilator functions are activated by acetylcholine (366).

Reflex pressor responses to carotid chemoreceptor stimulation (short periods of anoxia or asphyxia) are also reversed (252, 286, 393, 408) but the pressor responses to clamping of the common carotid arteries and to stimulation of the central end of the severed vagus nerve are inhibited without reversal (295, 393, 408, 409). At least part of the residual pressor effect of carotid occlusion after

Dibenamine may be due to a simple mechanical alteration of the vascular bed. Dibenamine has recently been employed as a tool to define other factors involved in the observed differences in the degree of inhibition or reversal by adrenergic blockade of various cardiovascular reflexes (116).

In contrast to the ergot alkaloids (see II, D-2), central nervous system depression does not appear to be a significant factor in the inhibition of vasomotor reflexes by Dibenamine. That the drug is devoid of actions on autonomic ganglia (116, 286, 292) and on reflex pathways in the central nervous system (116) appears to be adequately established. Intra-carotid injection of  $\beta$ -haloalkylamines produces little observable effect, whereas intra-arterial injection produces local vasodilatation (408, 409), presumably by blocking adrenergic vasomotor tone. In addition, reflex cardiac compensation for acetylcholine induced or orthostatic hypotension is unaltered in animals and humans, although responses to compensatory vasomotor reflexes are blocked (158, 432). Dibenamine in doses tolerated by the intact animal has not been shown to have any direct effects on smooth muscle, either of the vascular system, gut, uterus or nictitating membrane.

Several other manifestations of reflex sympatho-adrenal discharge have also been shown to be blocked by Dibenamine (286). The increases in erythrocyte and mononuclear leucocyte counts induced by fright or struggle are abolished, presumably because reflex contraction of the spleen and other blood reservoirs is prevented. The pilomotor response of cats to cold and fright as well as to electrical stimulation of the abdominal sympathetic chain is also abolished. In unanesthetized dogs, excitement may cause a fall in blood pressure for some time after Dibenamine administration (423).

The mydriasis induced in cats by dim light is moderately inhibited and that in response to cervical sympathetic stimulation is largely abolished (286). In man Dibenamine-induced miosis is more marked (see 183), probably because of a larger sympathetic component in the control of the iris. In contrast to the effects of the ergot alkaloids (see II, D-1), the miotic effect of Dibenamine appears to result from adrenergic blockade rather than from direct stimulation of smooth muscle. Except for certain differences in the sweating response, it appears that Dibenamine in adequate dosage may produce a typical Horner's syndrome. The use of Dibenamine offers interesting possibilities for evaluating the role of adrenergic stimuli in other ocular functions. Preliminary observations indicate that Dibenamine does not significantly alter accommodation (75).

Dibenamine blockade of epinephrine stimulation of the nictitating membrane develops more slowly (295, 373) and with small doses of the agent is less complete than blockade of the pressor response (295). This suggests a greater resistance of nictitating membrane than of vascular smooth muscle to blockade, or a poorer penetration of blocking agent into the former. Ocular smooth muscle has been shown to have a similar but much greater resistance to the effects of Priscol (see III, A-1).

It was early pointed out that Dibenamine more readily blocks responses to circulating epinephrine than those to direct sympathetic nerve activity (286).

However, it has since been contended that the failure of Dibenamine to reverse pressor responses to bilateral occlusion of the common carotid arteries and to electrical stimulation of the central stump of the severed vagus nerve indicates that the agent has little "sympatholytic" effect, *i.e.*, does not block responses to sympathetic nerve activity (408)

Because of the complexity of cardiovascular responses in the intact animal, more convincing evidence regarding the ability of Dibenamine to block sympathetic nerve activity is found in studies on the nictitating membrane of the cat. Such studies have reaffirmed the fact that responses to circulating epinephrine and norepinephrine (292) and to liver sympathin E (373) are more readily inhibited than those to nerve stimulation. However, they have also clearly demonstrated the ability of Dibenamine (292, 373) and several other members of the series (295) to inhibit the response of the nictitating membrane to cervical sympathetic nerve stimulation. The marked inhibition of responses to short periods and low frequencies of stimulation indicates that the responses of directly innervated cells are readily blocked (292). When combined with the many examples of blockade of the effects of reflex sympatho-adrenal discharge mentioned above, these observations warrant the conclusion that the  $\beta$ -haloalkylamine blocking agents are highly effective against most excitatory effects of sympathetic nerve activity as well as against responses to circulating sympathomimetic agents.

*3 Other "excitatory" responses* Dibenamine has been shown to block and occasionally to reverse the epinephrine-induced contraction of the non-pregnant rabbit uterus in both *in vitro* and *in vivo* experiments (286). This blocking action has been confirmed by Acheson and Farah (4) who have also studied the quantitative aspects of this inhibition with very instructive results (see I, C-2, C-3). Epinephrine-induced contraction of the isolated seminal vesicle of the guinea pig is similarly inhibited (281).

All the actions of Dibenamine mentioned above involve blockade of so-called E (excitatory) effects of epinephrine or sympathin on smooth muscle. Three points of blockade involving tissues other than smooth muscle have been established. Effective Dibenamine blockade has been demonstrated for adrenergic salivary secretion (404) and sweating (183, 156, 258), for the anti-curare action of epinephrine on the myoneural junction of skeletal muscle (249) and for the arrhythmia-inducing action of adrenergic stimuli on the sensitized myocardium (see I, A-5). The complete blockade of salivary secretion in response to cervical sympathetic nerve stimulation is not surprising in view of the many known pharmacological similarities between smooth muscle contraction and salivary secretion. Dibenamine inhibition of basal sweating (at least in certain areas) and of Neo-Synephrine-induced sweating may provide a useful tool in answering the controversial question concerning the extent to which adrenergic stimulation is involved in the normal sweating pattern (see 156).

Dibenamine prevents the anti-curare action of epinephrine but not that of KCl (249). Some specific, non-vascular action is apparently involved, and a careful study of this phenomenon may help to elucidate the poorly understood role of

adrenergic factors in skeletal muscle and perhaps also in autonomic ganglionic function

4 *Cardiac responses* It was early reported that Dibenamine fails to inhibit the chronotropic and inotropic actions of epinephrine (286, 293, 298, 408), circulating sympathin (286) and reflex and direct sympathetic nerve stimulation (116, 373) on the mammalian myocardium, and these observations have been confirmed *in vitro* (5) and in unanesthetized animals (432) and man (158, 183). The tachycardia induced by epinephrine is usually exaggerated because of the absence of reflex vagal slowing. In contrast to the consistent agreement of the above observations certain reports have suggested that Dibenamine (313) and its 1-naphthylmethyl and 2-biphenoxyethyl congeners (409) inhibit the chronotropic action of stellate ganglion stimulation and epinephrine respectively. No definite explanation of these discrepant observations can be made on the basis of the very limited data supplied in the reports. However, non-adrenergic factors must have been involved. In one series of experiments (313) nitroglycerine and papaverine produced an even greater "blockade" than Dibenamine. In addition, repeated experiments on dogs employing 1-naphthylmethyl and 2-substituted-phenoxyethyl derivatives of Dibenamine have uniformly shown these compounds to produce cardiac effects parallel to those of Dibenamine (293, 295). However, two non-specific actions of all active  $\beta$ -haloalkylamines in anesthetized animals have been found to give an apparent inhibition of epinephrine tachycardia. These are (A) a reduction in body temperature due to peripheral vasodilatation, which reduces the tachycardia in response to epinephrine and (B) a decrease in blood pressure which increases the control rate. Both of these factors reduce the extent of the increase in heart rate evoked by epinephrine, but no specific blockade of cardiac responses to adrenergic stimuli is involved.

The increase in cardiac rate after Dibenamine administration in anesthetized (298) or unanesthetized (423, 432) dogs persists for a much shorter period of time than the adrenergic blockade, and can probably be largely, if not entirely, explained on the basis of reflex compensation for the effects of Dibenamine on the peripheral vascular system. However, the marked increase in cardiac rate observed in schizophrenic patients given Dibenamine (258) may be a more complicated phenomenon.

Cardiac acceleration has been widely employed as a test for sympathin E (see 67), however, the failure of Dibenamine to block such acceleration casts doubt on the similarity of this response to the excitatory responses of smooth muscle. Resistance of the myocardium to Dibenamine blockade may be considered as support for the conclusion of Ahlquist (10), that the chronotropic and inotropic responses of the mammalian myocardium to adrenergic stimuli are more properly classed with the inhibitory responses of most smooth muscle.

In contrast to responses of the mammalian myocardium, the chronotropic response of the amphibian heart to epinephrine is blocked and even reversed by Dibenamine and congeners (295). This difference in the response of mammalian and amphibian hearts to adrenergic blockade has also been noted with several other types of adrenergic blocking agents. Its basis is unexplained.

5 *Cardiac arrhythmias* Although members of the  $\beta$ -haloalkylamine series of adrenergic blocking agents fail to inhibit the chronotropic and positive inotropic effects of epinephrine on the mammalian myocardium, Dibenamine and all active congeners tested very effectively prevent the cardiac arrhythmias induced either by epinephrine alone in unanesthetized dogs (423) and humans (183) or by epinephrine in the presence of cyclopropane sensitization (299). In comparative studies (298) Dibenamine was found to provide much more effective protection than any of a variety of other agents tested except certain other members of the  $\beta$ -haloalkylamine series (293, 295). Dibenamine also inhibits ventricular fibrillation induced by epinephrine after myocardial sensitization by other volatile hydrocarbons (135) and by DDT (295). Fibrillation after DDT is somewhat more difficult to block with Dibenamine than the arrhythmias induced by epinephrine in the presence of most other sensitizing agents. Dibenamine has also been found to prevent effectively "spontaneous" arrhythmias in surgical patients under deep cyclopropane anesthesia (283), a fact which suggests that endogenous epinephrine or sympathin is the cause of these arrhythmias. In both man and animals complete suppression of arrhythmias was found to require larger doses of blocking agent than are necessary to reverse the pressor response to injected epinephrine.

Studies of the mechanism of Dibenamine protection against cyclopropane-epinephrine cardiac arrhythmias have revealed at least two important components. One is a direct protection of the myocardium which is complete only with doses of Dibenamine larger than those required to reverse the pressor response to injected epinephrine (283, 293, 298), but which may also be dissociated from the peripheral blockade at lower doses (135). The other factor is protection of the myocardium against the stress of increased systemic arterial pressure which would result from the peripheral effects of epinephrine in the absence of blocking agent (269, 293). The sensitizing effect of elevated systemic arterial pressure which permits arrhythmias to occur in the presence of Dibenamine is cumulative and dependent upon the absolute pressure against which the left ventricle must work, rather than upon the magnitude of change in pressure (293), however, the rate of pressure change appears to play a significant role (269).

In addition to its adrenergic blocking action, Dibenamine has certain transient direct effects on the myocardium which are shared by its adrenergically inactive hydrolysis product 2-dibenzylaminoethanol. One of these effects is "quinidine-like." These agents decrease the sinus rate of the isolated rabbit auricle and the maximum rate at which the auricle will follow electrical stimulation (5). A transient increase in the threshold for electrically-induced fibrillation has also been noted (291). Dibenamine (prior to the onset of its adrenergic blocking action) and several adrenergically inactive congeners may actually sensitize the cat myocardium to epinephrine-induced arrhythmias (295). In addition, both Dibenamine and 2-dibenzylaminoethanol sensitize the dog heart to fibrillation after coronary occlusion (295). It is quite possible that these transient non-adrenergic myocardial effects of Dibenamine are involved in the cardiovascular collapse and death which may result from rapid intravenous injection of large doses.

It may be safely concluded that the non-adrenergic actions of Dibenamine on the myocardium are unimportant in protecting the heart against cyclopropane-epinephrine arrhythmias. These non-specific actions last only an hour or two, whereas the major protection against arrhythmias persists for 24 hours (293) and may be detected for as long as 8 days (423). The direct effects are also shared almost equally by 2-dibenzylaminoethanol which provides little or no protection against cyclopropane-epinephrine arrhythmias (293, 298).

6 *"Inhibitory" responses* In contrast to its effectiveness in blocking adrenergic excitatory responses, Dibenamine appears to be ineffective against inhibitory, metabolic and central nervous stimulant actions of sympathomimetic agents (286). The relaxation of rabbit, guinea pig and rat ileum produced by epinephrine *in vitro* is unaltered by concentrations of Dibenamine in the same range (1.1,000,000 to 1 15,000,000) as those blocking excitatory responses of smooth muscle (281, 286), this lack of effect has been confirmed by a study of intestinal activity in unanesthetized dogs (335). Much higher concentrations do produce a reduction in the epinephrine-induced relaxation (281), but this effect is probably non-specific, responses to acetylcholine and  $\text{BaCl}_2$  are almost equally inhibited. Epinephrine-induced relaxation of the non-pregnant cat uterus *in situ* is unaltered (286), but epinephrine-induced contraction of the rabbit uterus is readily inhibited both *in vivo* and *in vitro* (4, 286). A reported reversal of the bronchodilator actions of epinephrine and norepinephrine (360) is contrary to the usual lack of effect of Dibenamine on inhibitory responses. Unfortunately, the report in question provides no data regarding the concentrations of Dibenamine required or the magnitude of the effect, factors critical for an evaluation of the results.

It is evident that the reversal of the pressor response to epinephrine depends upon the inability of Dibenamine to inhibit adrenergic vasodilatation, this is emphasized by the lack of effect of Dibenamine on the depressor response to Isuprel (N-isopropyl-norepinephrine) (293, 295, 414). As expected, Dibenamine does not alter coronary blood flow or inhibit the increase in flow induced by epinephrine (232). The reported action of di- $\beta$ -chloroethyl derivatives of Dibenamine in inhibiting the depressor effects of epinephrine and Isuprel (197) is probably a reflection of the greater non-specific toxicity of these compounds which are true nitrogen mustards. Properly selected doses of N-benzyl-N,N-di( $\beta$ -chloroethyl)amine produce a typical Dibenamine-type reversal of the pressor response to epinephrine but the Isuprel depressor response is unaltered. Larger doses cause a reduction in the depressor response to Isuprel, but the response to methacholine is even more reduced, a fact which indicates a non-specific toxic action (295).

7 *Central nervous system and metabolic responses* Dibenamine fails to prevent epinephrine-induced hyperventilation in animals (286) or man (183). The increased activity caused by certain sympathomimetic amines in mice (281) and the "twittering" of chicks evoked by the same agents (78) are also unaltered. Dibenamine causes little reduction in the hyperglycemic response to epinephrine in animals (286, 300) and man (183), but certain congeners, notably those with 1-naphthylmethyl substituents, are quite active in this regard (300). It is pos-

sible that a slight glycemic-blocking action, detectable in studies on larger groups of animals (147) may extend through the entire series. Because Dibenamine inhibits local vasoconstriction and thus permits epinephrine to be more rapidly absorbed, small changes in the maximum glycemic response after subcutaneous administration of epinephrine are difficult to evaluate. Studies employing epinephrine administered intravenously will be necessary to determine whether massive doses of Dibenamine specifically inhibit the glycemic response to epinephrine. A report (32) that the BMR is reduced by Dibenamine does not provide adequate evidence upon which to base an interpretation. It is also difficult to evaluate the significance of an increased sensitivity to anoxia noted after Dibenamine administration (101), certain other adrenergic blocking agents have been observed to have an opposite effect (106).

Dibenamine does not prevent the prolonged "lighting" of fireflies induced by amphetamine (365). Whether this response to amphetamine should be classified as metabolic or central is not clear.

*8 Specificity* The specificity of the adrenergic blocking action of Dibenamine appears to be greater than that of other classes of adrenergic blocking agents studied to date. Dibenamine is ineffective against non-sympathomimetic smooth muscle stimulants. Cholinergic stimulation of the nictitating membrane (292), acetylcholine and histamine vasodepression (295, 414, 432), morphine stimulation of the intestine in unanesthetized dogs (335), acetylcholine and barium stimulation of rat intestine (281), and the pressor effects of ergotamine (217, 295), angiotonin (284, 432) and posterior pituitary (295, 408, 414, 432) are not blocked by doses of Dibenamine well above those which are effective against most adrenergic excitatory responses. The reported reversal of the pressor effect of KCl in intact animals (241) is best explained as an effect of Dibenamine on the sympatho-adrenal discharge evoked by potassium. Dibenamine also fails to block the direct vasoconstriction elicited by angiotonin and nicotine (155) in the L wen-Trendelenburg frog-limb perfusion preparation. Dibenamine has a slight antihistaminic action and some congeners are very potent in this regard (239, 290, 292). It is of interest that in a series of over 75  $\beta$ -haloalkylamines (290) no compound among the most effective antihistaminics failed to show adrenergic blocking activity. An incomplete report of inhibition of the depressor effect of stimulation of the peripheral end of the severed vagus by both Dibenamine and Priscol (255) implies some cholinergic blocking action. However, this action of Dibenamine has not been substantiated. Doses of 20 mgm/kgm have been found to alter neither the depressor nor the decelerator responses to standardized electrical stimulation of the vagus in dogs (295).

### *B Actions other than adrenergic blockade*

*1 Local actions* The primary side-effects of Dibenamine are local tissue damage after subcutaneous, intramuscular or intraperitoneal administration, and stimulation of the central nervous system (280, 286). The local tissue toxicity is dependent upon the  $\beta$ -haloalkyl grouping and reflects the relationship of these compounds to the nitrogen mustards. Local damage to vital abdominal organs

is largely responsible for the much greater toxicity of Dibenamine after intraperitoneal than after subcutaneous or slow intravenous administration (286) That intraperitoneally administered Dibenamine constitutes a considerable stress is indicated by the sharp drop in adrenal ascorbic acid which has been noted after such administration (394)

The systemic toxicity of the Dibenamine-type blocking agents is much less than that of the nitrogen mustards (about 1000 times less in the case of Dibenamine administered subcutaneously) Three factors are of importance in reducing the toxicity of these compounds (288), the presence of only a single  $\beta$ -haloalkyl group, a decreased aqueous solubility and a specific action of the aromatic grouping on the reactivity of the  $\beta$ -haloalkyl moiety (see I, D-2) Compounds with a single  $\beta$ -haloalkyl substituent fail to damage hemopoietic tissue even after prolonged administration

Prolonged daily subcutaneous administration of several times the blocking dose of Dibenamine and several congeners (281, 286) causes only a slight reduction in the growth of young rats and no histologically detectable organ damage (289) Oral administration of doses below about 1 gm /kgm appears not to produce detectable damage to the gastrointestinal tract However, doses necessary to produce an adequate block after oral administration may cause nausea and vomiting in humans (183, 337, 379, 424) Local irritation is probably a significant factor in this reaction, as only minimal side effects accompany the production of extensive blockade when the drug is administered intravenously with care (158, 258)

*2 Central nervous system* Stimulation of the central nervous system by Dibenamine in animals is manifest in hyperventilation, analepsis and even convulsions (280, 286, 298, 358) In humans, mild stimulation is frequently expressed as a specific loss of time perception (183, 337) This effect has been attributed to temporal lobe excitation (337), but the localization does not appear to be conclusively established Nausea and vomiting may also occur after intravenous administration (183, 337), particularly if it is rapid, probably on the basis of direct medullary stimulation Nausea and vomiting are unrelated to the adrenergic blockade Central nervous system excitation develops earlier and terminates long before the adrenergic blockade It is elicited even more markedly by the hydroxyl derivatives (hydrolysis products) of various members of the series (280, 286, 287) The central stimulant action of Dibenamine is accentuated by rapid intravenous injection, indeed, the intravenous toxicity may vary by as much as 1000% with variations in the rate of injection (286)

*3 Kidney* On the basis of experiments on a single dog, Ogden (303) reported that Dibenamine produced a marked reduction in renal plasma flow and glomerular filtration, and histological evidence of renal cortical necrosis Histological studies (289) carried out prior to clinical trial of Dibenamine failed to reveal any renal damage in rats, even in animals dying from the chronic administration of massive doses However, because of the expanding clinical study of Dibenamine, a detailed attempt was made to confirm the reported renal damage in dogs (411) Six weekly intravenous injections of 20 mgm /kgm Dibenamine



failed to produce any significant renal damage, as measured by glomerular filtration and renal plasma flow, even when each injection was completed within one minute to produce maximum side-effects including prostration of 24 to 48 hours duration. In none of the animals studied were persistent toxic effects of any kind noted. The above discrepancy in results remains unexplained, but the reported toxicity would appear to be most readily accounted for by some factor in the technic employed. The absence of any report of renal damage after fairly extensive clinical use of Dibenamine appears to confirm the lack of renal toxicity.

### C Locus and mechanism of action

1 *Locus of blockade* Studies on the locus and mechanism of action of members of the Dibenamine series of blocking agents have revealed certain factors which distinguish this group of compounds from previously studied blocking agents. On the basis of both *in vitro* and *in vivo* experiments it was pointed out that Dibenamine does not affect the alteration or destruction of epinephrine, does not inhibit the release of sympathin E and does not significantly alter the responsiveness of sympathetic nerves to electrical or reflex excitation (286, 292). The agent has also been shown to inhibit adrenergic functions directly rather than by a potentiation of antagonistic cholinergic or histaminergic responses (286, 292).

Quantitative studies on the Dibenamine blockade of the response of the cat nictitating membrane to cervical sympathetic nerve stimulation (292) have indicated that alterations in the permeability of the smooth muscle cells to sympathin is not a significant factor in the blocking action. Inasmuch as Dibenamine-treated cells may contract normally in response to acetylcholine, methacholine, ergotamine, nicotine, barium, posterior pituitary and angiotonin (see I, A-8), it is also apparent that Dibenamine does not paralyze the contractile mechanism of smooth muscle cells. By a process of exclusion it must therefore be concluded that Dibenamine specifically blocks some step in the process of excitation by sympathomimetic agents which is not necessary for stimulation by other substances, this step is interposed between penetration of the cell by the exciting agent and the actual process of contraction. The cellular material or process involved in this step has come to be referred to as the specific "receptor substance" or "receptor process," terms which only thinly disguise the fact that almost nothing is known regarding the true nature of this important step in the activation of effector cells.

2 *Type of blockade* Two distinguishing features of the Dibenamine type of blockade are its completeness and its duration. Extensive blockade may persist for three to four days after a single injection (183, 241, 286, 423), and residual effects after even longer periods of time have been reported (183, 423). It has also been observed (4, 281, 290) that the blocking actions of  $\beta$ -haloalkylamines *in vitro* are not reversed even after prolonged washing.

Studies of other series of blocking and exciting agents (acetylcholine-atropine (76), ergotamine-epinephrine (265) and diphenhydramine-histamine (413)) indi-

cate that antagonism occurs at a definite ratio for each pair of agents. This ratio, which varies somewhat with different test objects, implies a dynamic equilibrium of the blocking and exciting agent with some specific grouping or locus in the cell. In blockade of this type "effectiveness" and "potency" are essentially the same.

In the case of Dibenamine, however, it has been shown that once an adequate block has been established it cannot be overcome by massive adrenergic stimulation. This has been demonstrated for contraction of the cat nictitating membrane (292) produced by epinephrine, norepinephrine and cervical sympathetic stimulation, for the pressor action of epinephrine (4, 286) and other sympathomimetic amines (294) in cats, for contraction of the isolated rabbit uterus by epinephrine (4, 281) and for vasoconstriction by epinephrine in the L wen-Trendelenburg frog-limb perfusion preparation (155). Such findings cannot be explained on the basis of equilibria between mediator and blocking agent for some specific receptor. In this type of blockade "effectiveness" is not a function of "potency."

*3 Steps in the production of adrenergic blockade.* The prolonged action of the  $\beta$ -haloalkylamine blocking agents might result from persistence of active drug in the body or from some early action of the drug which is only slowly reversed. The blocking action of the  $\beta$ -haloalkylamines is apparently exerted through highly reactive intermediates formed in the body (sec I, D-1). These intermediates react rapidly and competitively with thiosulfate, and this reaction has been employed to determine the persistence of active drug in the body. If a high concentration of thiosulfate is maintained *in vivo* during the period of intermediate formation, adrenergic blockade never develops. However, if the thiosulfate concentration is allowed to fall before the completion of the reaction, blockade subsequently develops. In this manner, it has been possible to determine that the sojourn of effective amounts of Dibenamine in the body is approximately 12 to 18 hours (287). This relatively long period is presumably due to the high lipid solubility of the drug, which allows storage in fatty tissue, but it is still far short of the observed duration of blockade. The persistence of blockade beyond the period during which effective concentrations of active drug are present in the body suggests a destruction or prolonged inactivation of something in the cell which is essential for excitation by adrenergic stimuli.

The completeness and "non-equilibrium" character of the adrenergic blockade produced by the  $\beta$ -haloalkylamines also indicates a relatively irreversible inactivation of many or all of the cellular loci at which adrenergic stimulation must occur. However, it has been observed that in the period during which the Dibenamine blockade is developing *in vivo* (a period of at least 1½ hours) an equilibrium between epinephrine and Dibenamine does exist (288). Epinephrine administration at this time definitely reduces the effectiveness of Dibenamine. A similar equilibrium factor, manifested as a decreased sensitivity to epinephrine, has been observed in uterine strips treated with Dibenamine *in vitro* (4). From the above observations it may be concluded that the Dibenamine blockade develops in two stages. First, an approximation of Dibenamine to its site of

action This is probably at or near the site of action of epinephrine and consequently a state of competitive equilibrium may exist Second, an actual covalent chemical bonding of the blocking agent to some grouping near its site of action which prevents further equilibration with sympathomimetic agents and accounts for the completeness and duration of the blockade (288)

Acheson and Farah (4) observed occasional rabbit uterus preparations in which only the first step of this sequence appeared to occur, *i e*, the maximum control response could still be elicited if high concentrations of epinephrine were employed These "aberrant" preparations were exposed to Dibenamine for the same period of time as others which exhibited a clear reduction in the maximum possible response (non-equilibrium blockade) The basis for the difference is not clear, but it may well involve factors (tissue pH, etc) affecting the intermediate reactions of the  $\beta$ -haloalkylamines which are necessary for stable bonding (see I, D-1)

#### *D Relation of chemical structure to adrenergic blocking activity*

*1 Blocking reactions* The completeness and duration of the Dibenamine-type blockade are most readily explained on the basis of *in vivo* coupling with sulfhydryl, amino, imidazole or carboxyl groups Such reactions have been conclusively demonstrated for various  $\beta$ -haloalkylamines in connection with chemical warfare work on the nitrogen mustards (128, 129) They are dependent upon the formation of highly reactive ethylenimmonium or vinyl intermediates (288, 380, 381) The activity of members of the  $\beta$ -haloalkylamine series of adrenergic blocking agents has been shown also to depend upon intermediate formation (287, 296) Thiosulfate reacts rapidly and competitively with both immonium and vinyl intermediates formed from nitrogen and sulfur mustards, but not directly with the parent compounds (see 288) Prior administration of thiosulfate prevents the typical blocking action of even large doses of the  $\beta$ -haloalkylamines, although thiosulfate does not alter the blockade once it is established This indicates that these agents do not produce their blocking effect before the formation of intermediates Inasmuch as the final hydrolysis products are uniformly inactive, it may be concluded that the substances immediately responsible for adrenergic blocking activity are the intermediate transformation products The rather slow production of active intermediates *in vivo* probably also explains the slow onset of action observed even after intravenous administration

Available information indicates that all tertiary  $\beta$ -haloalkylamines probably undergo qualitatively similar intermediate formation and reaction, but it is also apparent from chemical studies (see 288) that other substituents on the amine may significantly alter the reactions of the  $\beta$ -haloalkyl moiety

*2 Chemical requirements for activity* A detailed study of 190 compounds related to Dibenamine (288) has revealed certain very specific requirements for adrenergic blocking activity These may be briefly summarized as follows

(1) The compound must be a tertiary amine All secondary amines tested were found to be inactive

(2) The compound must have a  $\beta$ -haloalkyl group capable of the formation

of an active intermediate. All substitutions for the halogen were found to result in inactivation, as does removal of the halogen to the  $\gamma$ -position.  $\beta$ - $\gamma$ -unsaturation which would also inhibit the formation of intermediates, completely inactivates otherwise active  $\beta$ -haloalkyl compounds.

(3) The compound must include an unsaturated ring structure attached to the nitrogen in such a way that hyperconjugation will act to stabilize the reactive intermediates formed. Substitutions on the unsaturated ring lead to loss of activity in proportion to the extent to which conjugation is inhibited. For example, a 4-chlorine substitution on the phenyl ring of N-benzyl-N-ethyl- $\beta$ -chloroethylamine results in an 80% loss of activity, whereas a 3-chlorine substitution does not reduce and may even slightly increase activity because it favors hyperconjugation. In the presence of one  $\beta$ -haloalkyl and one suitable unsaturated grouping the nature of the third substituent on the nitrogen usually has only a minor influence on activity.

(4) The unsaturated ring of the compound must satisfy certain steric requirements. The exact limits of these requirements are not clear, but substitutions which would tend to be out of the plane of the ring appear to produce inactivation.

Although the above structural requirements for activity in the  $\beta$ -haloalkylamine series cannot be considered as definitive, they have provided a practical and accurate basis for directing the synthesis of a large number of new compounds. In addition, these requirements point to certain very interesting theoretical considerations. In the past, discussions of structure-activity relationship in series of biologically active compounds have centered almost entirely upon molecular morphology, *i.e.*, steric factors. The general form of the molecules or the distances between particular atoms or radicals have been the usual considerations. In the  $\beta$ -haloalkylamine series of blocking agents, however, attention has been focused upon a very different property of the molecule, *i.e.*, its chemical reactivity. From the evidence presented above it may be concluded that the specific adrenergic blocking reaction, as distinct from whatever other reactions are involved in the toxicity of the nitrogen mustards, requires a stabilization of the reactive intermediate. Why this should be necessary can at the moment be only a matter of speculation, but it is apparent that the hyperconjugation required for activity will supply sufficient stabilization energy to alter markedly the reactions of the intermediates involved in the production of the blockade.

In addition to the large series of  $\beta$ -haloalkylamines discussed above, several members of the group have been reported separately (197, 240). These reports also have included a few compounds with minor alterations of structure (different length alkyl chains as the third (non-critical) substituent and different substitutions in the 2 position of the phenoxyethyl substituent) which were not included in the larger series. When corrected for certain errors due to inadequate testing methods (see I, D-3), the reported activities of these additional compounds in no way alter the above conclusions regarding the structural requirements for activity.

*3 Relative potencies* Determinations of the relative potencies of various members of the Dibenamine series are still unsatisfactory. Although the  $\beta$ -haloalkylamines have been shown to exert their blocking action by all routes of administration (286), most routes are not suitable for quantitative tests. Most of the compounds, particularly those with a favorable therapeutic index, are quite insoluble in aqueous media (288). Because of this insolubility and because of varying but quite rapid rates of decomposition (288), oral administration (240) seems to provide an inadequate basis for quantitative comparisons. The factor of solubility also limits the accuracy of tests involving subcutaneous administration, use of this route as well as adherence to a rigid time schedule probably accounts for the reported inactivity (197) of several compounds which are fully active after intravenous administration (288). The varying rates at which blockade develops (288) and the effect of epinephrine in reducing the degree of blockade during its initial stages (see I, C-3) also represent obstacles to accurate comparisons of potency.

However, certain general conclusions may be drawn concerning potency. It is apparent from all tests (197, 240, 288, 295) that Dibenamine is not the most potent member of the series. The compound N-ethyl-N-(1-naphthylmethyl)- $\beta$ -chloroethylamine possesses a relatively high potency, although much of the observed advantage when administered orally (240) or subcutaneously (197) is undoubtedly due to its high aqueous solubility (which is correlated with a high toxicity (288)). When tested for peak effect after intravenous administration in cats, it appears to be somewhat less than twice as potent as Dibenamine (295). The most active compound studied to date is N-benzyl-N-(2-methylphenoxyethyl)- $\beta$ -chloroethylamine which is about 5 times as potent as Dibenamine when administered intravenously (295). It is of practical importance that this agent is also one of the least toxic members of the  $\beta$ -haloalkylamine series.

*4 Application to other fields* The reactions involved in adrenergic blockade may vary widely from one group of blocking agents to another. It is obvious that the above considerations of the mechanism of blockade cannot apply to members of such series as the ergot alkaloids, benzodioxanes or imidazolines. More subtle distinctions must also occasionally be made. For example, certain primary and secondary phenoxyethylamines without a  $\beta$ -haloalkyl group are adrenergic blocking agents (see VI, B-1). These compounds appeared to represent major exceptions to several of the chemical requirements for activity discussed above until it was noted (288) that the blockade produced is very different in character from that produced by Dibenamine, *i.e.*, it is very much shorter in duration and is essentially unaltered by the presence of thiosulfate.

The demonstration that precise chemical configurations are necessary for adrenergic blocking activity in the  $\beta$ -haloalkylamine series and the insight which this information has provided regarding the chemical reactions involved in the blocking action raise the possibility that other properties of these and related agents may yield to a similar analysis. It may be hoped that comparable studies of other properties, such as the destructive action of the nitrogen mustards on certain neoplasms (139, 144, 201, 332), may provide a theoretical basis for

syntheses leading to much-needed improvements in specificity. Current attempts to improve the specificity of the anti-neoplastic action of certain  $\beta$ -halo-alkylamines, *e g*, by attaching them to carcinogens (126), have been carried out with only a very limited knowledge of the chemical basis for the desired action and have met with little success.

## II ERGOT ALKALOIDS

Older work on the ergot alkaloids has been excellently reviewed by Barger (20, 21) and will be referred to here only as necessary to clarify more recent results. The first demonstrations of specific adrenergic blockade by any agent were those of Dale (85) and Sollmann and Brown (375) who employed crude or only partially purified ergot preparations. Since then the ergot alkaloids have been extensively investigated, but few basic observations have been made which were not indicated by the classical studies of Dale (86). The alkaloids employed by Dale in these experiments were not completely purified and identified. However, they appear to have been very similar to ergotoxine and his results will be considered as representative of the actions of that agent.

Unfortunately, so much emphasis has been placed on the adrenergic blocking activity of these compounds that their other pharmacological properties frequently have been overlooked with consequent misinterpretation of experimental results. The most important side-effects are direct stimulation of smooth muscle and complex excitant and depressant effects on the central nervous system. Under many experimental conditions these side-effects are prominent with doses smaller than are required to produce significant adrenergic blockade.

### *A Alkaloids lacking adrenergic blocking activity*

By 1930 the pharmacology of ergotamine and ergotoxine had been extensively studied and it was generally accepted that both the oxytocic and adrenergic blocking activities of ergot resided primarily in these alkaloids. However, in 1932 Moir (27) pointed out that crude extracts of ergot produced a more potent and rapid effect on the parturient uterus than any of the known alkaloids. Investigations designed to clarify this difference in activity resulted in the almost simultaneous isolation of the alkaloid ergonovine in four separate laboratories (see 216). In ergonovine the lysergic acid nucleus is attached to a simple amino alcohol (2-aminopropanol) rather than to a polypeptide chain as in members of the ergotamine and ergotoxine groups of alkaloids (see table I).

The earliest observations on the pharmacology of ergonovine (54, 345, 347) demonstrated it to be essentially devoid of adrenergic blocking activity. A slight reduction in the pressor response to injected epinephrine is observed with large doses (345), but the effect may well be non-specific. Ergonovine is reported to be somewhat more potent than ergotamine in stimulating the guinea pig uterus *in vitro*, but to be a less active vasoconstrictor (345, 347). It has a stronger pressor effect than ergotamine in anesthetized rabbits (345), although it is less pressor than ergotoxine in pithed cats (54). The latter observation is the better index of peripheral vasoconstriction as much of the pressor effect of

this agent, at least in cats, is due to stimulation of spinal vasomotor centers. Under certain conditions ergonovine may actually produce vasodilatation in the perfused cat limb (54). On a weight basis ergonovine has been reported to be twice as potent as ergotamine or ergotoxine in producing cyanosis of the cock's comb (346). The unexpected combination of an *increased* production of cyanosis (probably a rather accurate measure of direct vasoconstriction) accompanied by a *decreased* production of gangrene (54, 345, 347) does not appear to have been adequately studied or explained. Ergonovine is of importance in the field of adrenergic blockade only in control experiments designed to determine the extent to which the effects of other ergot alkaloids are due to side-effects. It has been employed too infrequently for this purpose.

TABLE I  
*Composition of the natural alkaloids of ergot\**

<b>I Ergotamine-group</b> 1 Ergotamine Ergotaminine 2 Ergosine Ergosinine	{ Lysergic acid NH <sub>2</sub> Pyruvic acid d-Proline	+ l-Phenylalanine  + l-Leucine
<b>II Ergotoxine-group</b> 3 Ergocristine Ergocristinine 4 Ergokryptine Ergokryptinine 5 Ergocornine Ergocorninine	{ Lysergic acid NH <sub>2</sub> Dimethyl-pyruvic acid d-Proline	+ l-Phenylalanine  + l-Leucine  + l-Valine
<b>III Ergobasine**</b> Ergobasinine	Lysergic acid	+ d-2-Aminopropanol

\* From Rothln (351)

\*\* Identical with Ergonovine

Methergine, a partially synthetic alkaloid (385), differs from ergonovine only by the presence of an additional methylene group, and appears to have pharmacological properties almost identical with those of ergonovine (218). This compound was selected for clinical use because it was found to be the most effective uterine stimulant among members of a large series of rather simple amides of lysergic acid (385).

One partially synthetic member of the ergonovine group deserves special mention because of its remarkable effects on the central nervous system (389). The diethylamide of lysergic acid has been descriptively termed "ein Phantastikum". In man oral doses as small as 10 to 30 micrograms cause marked and highly specific psychic responses. These include a state of fluctuating euphoria and depression and alterations in almost all sensory perception. The development of vividly colored and rapidly changing visual hallucinations is particularly characteristic. In view of the fact that the active *l*-isomers of all ergot alkaloids

possess powerful central nervous system actions (see II, D-2), the properties of lysergic acid-diethylamide may be considered merely as extensions of actions common to the group

In addition to evoking the above psychic effects, lysergic acid-diethylamide stimulates the isolated uterus only slightly less than ergonovine (385, 389) and produces marked analepsis in anesthetized animals. It is probably safe to assume that, in common with all other non-polypeptide ergot alkaloids studied, it is devoid of adrenergic blocking activity

### B Chemistry

Since the isolation of ergonovine, two major advances have been made in the chemistry of the ergot alkaloids, both in the laboratory of Dr Arthur Stoll. In 1943 it was reported that ergotoxine is really a complex of three alkaloids (386) which were given the names ergocornine, ergocristine and ergokryptine. Ergocristine had been isolated and characterized in 1937 (384), but at that time it was not recognized as a component of "ergotoxine." In common with ergotamine, but in contrast to ergonovine, these compounds include a polypeptide moiety (see 388). The terminal residue of the peptide chain is *D*-proline in all members of both the ergotamine and ergotoxine groups (see 383). Members of the ergotoxine complex contain dimethyl-pyruvic acid instead of pyruvic acid as in ergotamine and ergosine and differ from each other only in one amino acid residue (388) (table I). However, they do differ very significantly in their adrenergic blocking activity (55, 351, 353). The observed effect of variations in amino acid constitution upon blocking activity suggests that the synthetic substitution of still other amino acids might prove to be a fruitful line of endeavor.

A second major advance in the chemistry of the ergot alkaloids was the partial reduction of all members of the ergotamine and ergotoxine groups to form their dihydro derivatives (387). Hydrogenation decreases the ability to stimulate smooth muscle and increases the adrenergic blocking activity of all natural alkaloids. Specific properties of the reduced alkaloids will be considered together with comparable properties of the parent compounds.

### C Adrenergic blocking action

1 "Excitatory" responses. As noted above, all ergot alkaloids with significant adrenergic blocking activity contain a polypeptide side-chain. Although their potency relationships follow a general pattern in various tests, with ergotamine the least, and ergocristine and ergokryptine the most potent, this order of activity is not inviolable. Ergocornine is significantly more active than ergotamine when tested against epinephrine-induced contraction of the guinea pig seminal vesicle *in vitro* (55, 351), but the relationship is reversed when the agents are tested for their ability to antagonize the effects of epinephrine on the rabbit uterus (351, 353).

The adrenergic blocking potency of the reduced alkaloid is always greater than that of the parent compound. *In vitro* tests on the guinea pig seminal vesicle



and rabbit uterus (55, 351, 353), although not providing identical potency ratios, allow agreement on an order of increasing potency dihydroergotamine, dihydroergocornine, dihydroergocristine and dihydroergokryptine. No quantitative comparisons of their effectiveness in blocking cardiovascular responses to adrenergic stimuli in intact animals are available, but dihydroergotamine is definitely less active than the derivatives of members of the ergotamine group (295). Some workers have experienced difficulty in obtaining clearcut reversals of the epinephrine pressor response even with rather large doses of dihydroergotamine (304, 305).

The dihydro ergot alkaloids appear to have a relatively short duration of action *in vivo* when tested for antagonism of the pressor (224, 295, 350, 351) and rabbit uterine responses (351) to epinephrine. This duration of action might have been anticipated from the ease with which these compounds are "washed out" of *in vitro* preparations (55, 353). The adrenergic blocking action of both the natural alkaloids and their dihydro derivatives persists for a considerably shorter period of time than their other pharmacological actions.

Early experiments of Dale (86, 87) demonstrated that large doses of the ergot alkaloids produce a blockade which is effective against strong adrenergic stimuli and this property is shared by their dihydro derivatives (295). With the exception of the  $\beta$ -haloalkylamines, all other series of adrenergic blocking agents produce a less complete and effective blockade. Larger doses of ergotamine and ergotamine (see 24, 86) are required to inhibit the pressor response to splanchnic stimulation than that to injected epinephrine. In the absence of evidence to the contrary it may be assumed that the dihydro derivatives behave in a qualitatively similar manner. Considerably larger doses of these agents are required to block the response of the cat's nictitating membrane to cervical sympathetic nerve stimulation than to block the response to injected epinephrine (295).

The ergot alkaloids, particularly the dihydro compounds, markedly increase the lethal dose of intravenously administered epinephrine (351). Ergotamine also reduces the amplitude and increases the rate of ureteral peristalsis *in vivo* (148). This effect could be due either to a blockade of adrenergic stimuli or to a direct musculotropic effect of the ergotamine (247). No effort was made to distinguish between these possibilities.

Jang (204) has pointed out that small doses of ergotamine sensitize the vessels of the rabbit's ear to epinephrine and the smooth muscle of the cat's nictitating membrane to sympathetic nerve stimulation. He emphasized the structural similarity between ergotamine and cocaine and suggested a common mode of action. However, the functional significance of this comparison may be questioned on the basis that yohimbine, phenoxyethylamines, benzodioxanes and other agents can produce similar sensitization.

Potentialization of pressor responses to sympathomimetic agents by the ergot alkaloids is enhanced by conditions which reduce their adrenergic blocking effectiveness (189). Such sensitization is particularly evident in the presence of barbiturate anesthesia which has long been known to inhibit the adrenergic

blocking action of the ergot alkaloids (see 66) It has been reported (224) that dihydroergotamine, in contrast to ergotamine, is effective in the presence of barbiturate anesthesia However, the relative reduction in potency in the presence of pentobarbital as compared to urethane anesthesia appears to be roughly the same for a number of natural and hydrogenated alkaloids (295) The blockade produced by dihydroergotamine in the presence of pentobarbital anesthesia thus appears to be merely one expression of the increase in potency induced by hydrogenation

It has been known for many years (321) that the ergot alkaloids may alter from depressor to pressor the response to amines such as N-ethyl- and N-isopropyl-norepinephrine and 3,4-dihydroxyephedrine, recent interest in Isuprel (N-isopropyl-norepinephrine) has led to a number of confirmatory studies (169, 217, 295) The original explanation for this phenomenon which postulated a greater blockade of inhibitory than of excitatory vascular responses by ergot now appears untenable The pressor response to depressor amines in the presence of an ergot alkaloid is definitely sympathomimetic in nature because it is reversed by the benzodioxanes and yohimbine and potentiated by cocaine (170) Little else is known regarding its characteristics Among the various natural and dihydrogenated alkaloids, "depressor reversal" potency is not parallel to adrenergic blocking potency (169, 295) Posterior pituitary produces a similar reversal (171)

**2 Cardiac responses** Dale (86) noted that the ergot alkaloids failed significantly to alter responses of the mammalian myocardium to adrenergic stimuli and this observation has been confirmed by many workers Dihydroergotamine (349) and other dihydro ergot alkaloids (348, 351) also fail to affect adrenergic chronotropic and inotropic cardiac responses in mammals, even when employed in massive doses In view of the consistent results of well-controlled animal experiments the apparent inhibition of epinephrine tachycardia in a few patients by dihydroergokryptine (121) must be attributed to extraneous factors, perhaps the antagonistic effect of central vagal activation It has been known for many years that the ergot alkaloids produce a marked cardiac slowing which is due to central vagal stimulation, the bradycardia persists after sympathectomy but not after vagotomy (273, 339) Cardiac slowing probably on the same basis, has been noted in almost all studies on the dihydro alkaloids

In contrast to their lack of effect on responses of the mammalian heart to adrenergic stimuli, the ergot alkaloids inhibit and even reverse the action of epinephrine on the frog heart (14, 295, 343) Changes in the ionic composition of the perfusion fluid readily alter this antagonism (222), a fact which may explain the failure of some workers to observe it (24, 154) Dihydroergotamine is slightly and dihydroergocornine is considerably more potent than ergotamine in inhibiting the chronotropic response of the isolated frog heart to epinephrine (295) Negative results obtained in experiments employing dihydroergotamine in a single concentration (39) are of questionable significance

**3 "Inhibitory" responses** Dihydro derivatives of the ergot alkaloids antagonize the inhibitory response of the isolated rabbit intestine to epinephrine (351)

in much the same way that ergotamine and ergotovine antagonize inhibitory responses (277, 308, 343, 344, 396). However, some workers have concluded that the ergot alkaloids do not produce a specific blockade of adrenergic inhibitory responses. This conclusion is based particularly upon the failure of these agents to block adrenergic inhibitory responses of the uterus of various species, particularly that of the non-pregnant cat (265, 344), and of the vascular musculature (86, 213). A report of inhibition of epinephrine-induced uterine relaxation by dihydroergotamine (39) is inconclusive. Only one dose of the blocking agent was employed and this simultaneously eliminated responses to histamine, ergonovine and ergotamine. The report fails to record the species employed. The dihydro derivatives as well as the parent alkaloids fail to block inhibitory vascular responses (349). Blockade of vasodepressor reflexes (343) does not constitute evidence of an antagonism of adrenergic inhibitory responses because of the known central depression of vasomotor reflexes by the ergot alkaloids (see II, D-2).

Intestine represents a much less suitable test object than vascular and uterine smooth muscle upon which to study adrenergic inhibitory responses. The abundance of intramural parasympathetic ganglia in the intestine and the known effect of the ergot alkaloids in potentiating cholinergic responses (86, 188, 237, 342) make it impossible to attribute alterations of epinephrine-induced intestinal relaxation to specific adrenergic blockade. Even the uterus of certain species, *e.g.*, guinea pig (7), show an apparent inhibition and reversal of epinephrine relaxation in the presence of eserine, *i.e.*, after the potentiation of cholinergic effects. Ergotamine definitely enhances the motility of the intact intestine (6, 356, 405). However, doses which alone are completely ineffective markedly potentiate the action of neostigmine (6), cocaine fails to inhibit motility under conditions where ergotamine produces a significant stimulant effect (405) and the response to ergotamine is blocked by atropine (356). Blockade of epinephrine-induced inhibition of the rabbit intestine and of epinephrine-induced contraction of the rabbit uterus is accomplished with essentially the same concentrations of the ergot alkaloids, but adequate data have not been presented to indicate that the potency ratios of the various alkaloids and their dihydro derivatives are the same when tested against excitatory and inhibitory responses. Much more experimental work will be necessary before it can be stated with any assurance that antagonism to inhibitory responses by ergot alkaloids represents a specific adrenergic blockade.

*4 Metabolic responses* The ergot alkaloids block epinephrine-induced hyperglycemia more effectively than do other adrenergic blocking agents (see 343). Dihydro derivatives of members of the ergotovine group also block this response (121, 350, 351), but the potency of this effect is not parallel to adrenergic blocking potency as determined by other tests (351). One report indicates that dihydroergotamine may be twice as effective as ergotamine in blocking the glycemic response to epinephrine in humans (378). It has been reported that adrenergically inactive ergonovine fails to block and may actually enhance the glycemic response to epinephrine (347), but the doses used were not specified.

Ergonovine in doses of 3.0 mgm /kgm subcutaneously provides effective blockade of the glycemic response to epinephrine in rabbits (300). The specificity and mechanism of the ergot blockade of the glycemic response to epinephrine are questioned by the activity of ergonovine, and by the fact that posterior pituitary causes a comparable reduction in the glycemic response to epinephrine, an effect which is additive with that of ergotamine (228). As in the case of blockade of inhibitory adrenergic responses, the available evidence is not adequate to allow a definite statement that inhibition of epinephrine-induced glycemia is due to a specific adrenergic blockade comparable to that produced in smooth muscle.

#### *D Actions other than adrenergic blockade*

**1 Smooth muscle** One of the most characteristic effects of the ergot alkaloids is a direct stimulation of smooth muscle in many organs (86). This effect was known long before the demonstration of adrenergic blockade by these agents (20, 21) and under most conditions it occurs with smaller doses of the natural ergot alkaloids than are required to produce adrenergic blockade. In the case of ergot alkaloids lacking a peptide substituent, uterine stimulation is prominent in the complete absence of adrenergic blocking activity (see II, A). It has been suggested that the stimulation of smooth muscle by the ergot alkaloids is "sympathomimetic" in nature, but the fact that it is unaltered by Dibenzamine (31, 217, 295) is strong evidence against this interpretation.

Hydrogenation produces a marked reduction in the uterine stimulant action of all the ergot alkaloids studied. The dihydrogenated alkaloids not only fail to cause contraction of rabbit or guinea pig uteri *in vitro* (305, 348, 350, 351) or induce labor in pregnant rats (305), but also tend to diminish uterine tone and activity and to inhibit the excitant effects of ergotamine and ergonovine on the uterus (348, 350, 351). Inhibition of the stimulant effect of the natural alkaloids may be related to the previously observed block by ergotamine of its own vasoconstrictor action (24, 237). The mechanism of this effect has not been studied, but it probably does not constitute proof of the "sympathomimetic" nature of smooth muscle stimulation by this agent. Hydrogenation may not completely eliminate oxytocic action, for the induction of labor pains in a pregnant woman given dihydroergotamine for migraine has been reported (398).

Hydrogenation also reduces but does not eliminate the vasoconstrictor actions of the ergot alkaloids, as measured by their pressor action in pithed cats (350, 351). Although dihydroergotamine is less vasospastic than the parent alkaloid it retains considerable constrictor activity in man (37, 38). Dihydroergocornine fails to evoke detectable vasoconstriction in anesthetized dogs (211) and plethysmographic studies (34, 37, 38) indicate that it has little or no direct vasoconstrictor action in unanesthetized man. Only in the pithed cat may a residual vasoconstrictor action of dihydroergocornine be detected (351). Elimination of the prominent vasospastic action characteristic of the natural ergot alkaloids may provide an opportunity to explain certain discrepancies between the actions of different groups of adrenergic blocking agents, *e.g.*, the greater inhibition of the vasopressor action of nicotine by ergotamine (86, 173) than by Dibenzamine

(408), 883F, 933F and yohimbine (174, 407) This difference may be unrelated to variations in adrenergic blocking activity and may depend upon differences in the peripheral action of nicotine upon relaxed (after Dibenamine) as compared to constricted (after ergotamine) vessels

Production of cyanosis and gangrene in the rat's tail or cock's comb is a prominent response to the natural ergot alkaloids, and the production of cyanosis in the cock's comb was for many years the official assay method for extract of ergot (see USP XII) The early development of a transient cyanosis is probably largely due to a direct vasoconstrictor action, but the basis for the late development of "thromboangitis" and gangrene (242, 425) is not clear It is not necessarily related to the extent of the initial cyanosis (see II, A) Although thrombosis is characteristic of the pathological picture in ergot gangrene, it is apparently of limited etiological significance because the incidence of gangrene is completely unaltered by heparin and dicoumarol (16) Hyperthyroidism definitely sensitizes rats and probably also human beings to the production of ergot gangrene (150) It is not clear why gangrene may develop several days after a single injection of ergot although the alkaloids appear to be destroyed very rapidly (223, 352) Dihydroergotamine has very much less tendency than ergotamine to produce gangrene (305), and the other hydrogenated alkaloids appear to have so little direct effect on the peripheral vascular system that thorough tests of their ability to produce gangrene have not been undertaken

It is well established that ergotamine may act as a direct coronary vasoconstrictor (213) It hastens the development of pain in patients with angina pectoris breathing an atmosphere low in oxygen (138) and may even cause anginal pain in susceptible patients at rest (398) The report of an increased coronary blood flow induced in man by dihydroergotamine (377) is based only upon electrocardiographic changes No pharmacological or physiological basis for such an action is apparent, and in view of the many other direct and indirect cardiac effects of the ergot alkaloids (see II, C-2, D-2 and VII, E), alterations in the electrocardiogram appear to provide an inadequate basis for adducing changes in coronary blood flow More reliable testing methods indicate that ergotamine and dihydroergotamine increase cerebral blood flow in the absence of changes in systemic arterial pressure (3), but an evaluation of this effect must await a full report of the observations It is possible that this effect on cerebral blood flow is involved in the reported protection against anoxia afforded by ergotamine (106) However, adrenergic blockade *per se* does not provide protection against anoxia, Dibenamine has been reported even to produce sensitization (101)

The ergot alkaloids cause mydriasis in rodents, in both sympathetically denervated and normal eyes (98), undoubtedly as a result of direct smooth muscle stimulation (22) The reaction has been well quantitated for ergotoxine and ergonovine (26), and similar tests on the dihydro alkaloids would be of considerable interest In the cat, miosis is produced by ergotamine and ergotoxine (86, 426), largely on the basis of a direct stimulation of the iris sphincter (426) In this case inhibition of sympathetic dilator tone may also be involved but

appears to be insignificant. The species differences in response of the iris to ergot alkaloids may be due to differences in the relative strengths of the dilator and constrictor muscles. The report that ergonovine produces mydriasis rather than miosis in the cat (345) unfortunately does not include experimental data upon which an evaluation of this unexpected result might be based. However, the evidence of hypothalamic stimulation seen with toxic doses of ergonovine (347) may provide a clue to the mechanism of this response.

The action of the ergot alkaloids in producing perforating gastric ulcers after both oral and intravenous administration (342, 347) has been inadequately studied. Vascular spasm and pylorospasm would appear to be possible etiological factors deserving of careful study. No theoretical basis for the therapeutic administration of ergotamine in peptic ulcer (103) is evident.

*2 Central nervous system.* The effects of ergot alkaloids on the central nervous system represent a highly complex mixture of stimulation and depression (see 339 for earlier work). Unfortunately, only limited data on the central effects of the dihydro alkaloids are now available. Their acute intravenous toxicity is significantly reduced as compared to the toxicity of the parent compounds (305, 350, 351), but the extent to which this is a measure of central nervous system action is not clear. Somnolence and general sedation are prominent among the signs of acute ergot toxicity in monkeys (420), and injection of ergotamine base into the third ventricle of cats leads to prolonged, and apparently normal sleep (190). The fall in blood pressure regularly noted in intact animals after intravenous administration of the dihydrogenated alkaloids has been attributed to direct depression of the vasomotor center and stimulation of the "vasodepressor" center (351). Elimination of this hypotensive effect by spinal cord section above  $T_6$  (35) provides convincing evidence of its central origin. A marked bradycardia is also produced by relatively small doses of the ergot alkaloids and their dihydro derivatives (36, 38, 121, 342, 348, 350). Ergonovine is about one-half as potent as ergotamine in producing this response (231). This bradycardia is due to a direct stimulation of vagal centers rather than to sympathetic blockade, because it persists after high spinal cord section (35) or sympathetic denervation of the heart and is abolished by vagotomy (273, 339). The central locus and the non-specificity of the depressor effects of the ergot alkaloids is well illustrated by the fact that very small doses of ergotovine injected intracisternally cause a marked fall in blood pressure and also inhibit the pressor response to KCl administered by the same route (107).

Depressant effects on the central nervous system adequately explain the fall in blood pressure, the orthostatic hypotension and the decreased vasomotor reflexes in human subjects given doses of the dihydro ergot derivatives which are inadequate to produce adrenergic blockade (36, 37, 38, 121). These observations suggest that hydrogenation increases the depressant action of the alkaloids on the central nervous system. However, the apparently greater central depressant effect of these derivatives, as measured by cardiovascular indices, may merely be due to their inability to evoke peripheral vasoconstriction. This problem would seem to warrant careful investigation by more direct methods.

than have been employed Ergonovine produces quite significant depression of vasomotor and respiratory centers in anesthetized animals, although it elicits an increase in pressure (347)

It has been reported (350) that hydrogenation reduces the ability of the ergot alkaloids to depress the respiratory center, but that it does not alter their vasomotor depressant action It is unfortunate that this report does not include or refer to data regarding the extent and significance of this differential effect Dihydroergotamine and dihydroergocornine produce less respiratory depression in anesthetized dogs than does ergotamine (211) However, all of the derived alkaloids, in the doses required to block responses to sympathetic nerve stimulation do produce marked respiratory depression in anesthetized cats (295)

Ergotamine produces a marked stimulation of somatic motor neurones (339), but the extent to which this action is shared by the dihydro alkaloids has not been determined Reports that dihydroergotamine is 6 to 8 times less emetic than ergotamine (348, 350) do not appear to have been substantiated The ratio has since been observed to be about 1:2 in puppies (305) and not greater than 1:2 in patients with migraine (398) Other dihydro derivatives also appear to have a strong emetic action in man (34, 36, 37, 121, 167), as little as 0.3 mgm of dihydroergocornine may produce vomiting (34, 36) A report of antagonism to apomorphine emesis in dogs by supra-emetic doses of dihydroergotamine is incomplete (224), but this antagonism appears to be due to brain-stem depression, ergotamine produces an exactly comparable effect (73) Ergotamine raises the electroshock seizure threshold in rabbits (162), an action which is obviously not due to adrenergic blockade because ergonovine has an equal effect

The ergot alkaloids rather specifically depress certain centers in the brain stem The effects of  $\text{CO}_2$  on blood pressure and respiration are readily inhibited, apparently by decreasing the responsiveness of medullary centers to direct stimulation by  $\text{CO}_2$  (235) Depression of the response to  $\text{CO}_2$  occurs with doses of ergotamine which do not inhibit chemoreceptor reflexes or prevent the vascular effects of epinephrine or splanchnic nerve stimulation Dihydroergotamine appears to be somewhat less effective than ergotamine in reducing medullary sensitivity to  $\text{CO}_2$  (110, 350) Other dihydro ergot alkaloids have not been tested for this action

Ergot alkaloids also inhibit vascular responses to carotid baroreceptor (110, 111, 112) and chemoreceptor (137, 252) reflexes in doses which do not significantly alter vascular responses to injected epinephrine or direct splanchnic nerve stimulation Dihydroergotamine appears to be about as effective as ergotamine in this respect, although it has been stated without the presentation of substantiating data, that dihydroergotamine and dihydroergocornine produce somewhat less depression of carotid sinus reflexes than does ergotamine (350) The complete dissociation of this inhibition of vasomotor reflexes on the basis of brain-stem depression from adrenergic blockade is emphasized by the fact that adrenergically inactive ergonovine is about one-half as potent as ergotamine in depressing responses to carotid sinus nerve stimulation (347)

Electrical recording from the splanchnic nerves has indicated that the flow of

impulses induced by carotid chemoreceptor stimulation is increased rather than decreased by ergotamine (137). This observation is difficult to interpret, but it does not appear to be evidence for a primarily peripheral site of action of the ergot alkaloids in altering cardiovascular reflexes. The frequently observed inhibition of reflex cardiovascular responses by the dihydro ergot alkaloids in man (see VII, A, D-3, E) is undoubtedly due to the central nervous system effects described above and not to a "sympatholytic" action as is so frequently stated.

An increased body temperature evoked by the administration of relatively pure ergot alkaloid preparations was noted early in the study of these compounds (see 22). This effect is probably unrelated to adrenergic blockade because it is also produced by adrenergically inactive ergonovine (26, 54, 345) and Methergin (218). It has recently been reported (348, 350, 351) that ergotamine and ergocristine cause hyperthermia while their dihydro derivatives produce hypothermia. However, the specificity of these differences in action is questioned by experiments which indicate that ergotamine produces a non-specific impairment of temperature regulation in rats, with hyperthermia observed at environmental temperatures above 28°C and hypothermia at low temperatures (56). Impaired temperature regulation after ergotamine was previously observed in cats (359). The absence of a specific effect of the ergot alkaloids on heat production or dissipation is suggested also by the observation that neither ergotamine nor dihydroergotamine significantly alters the hyperthermic response to dinitrophenol (127). Lack of adequate control of ambient temperature may be responsible for many of the divergent results which appear in the literature on this subject.

*3 Miscellaneous effects* The reported diuretic and antidiuretic properties of several ergot alkaloids (434, 435, 436) have not been re-evaluated with modern clearance techniques, and the alleged differences between various ergot preparations are inexplicable on the basis of published data. Ergotamine and dihydroergotamine inhibit both the internal and external secretions of the pancreas (149). The secretory response to secretin, but not that to epinine is inhibited. The reported inhibition of the bronchoconstrictor response to acetylcholine in the guinea pig by ergotamine (182) is difficult to interpret, particularly because there is evidence (see II, C-3) that the ergot alkaloids potentiate many responses to acetylcholine and vagal stimulation.

*E Fate and excretion* Attempts to elucidate the fate and excretion of the ergot alkaloids by the use of sensitive biological tests (adrenergic blocking action on the rabbit uterus or guinea pig seminal vesicle) (352) have provided primarily negative information. Urinary excretion is insignificant. Parenchymatous organs (particularly the liver) apparently contain larger concentrations of alkaloid than does the circulating blood. However, tests carried out at intervals between 5 and 60 minutes after intravenous injection were never capable of detecting a total of more than 5% of the administered alkaloid. Penetration of significant amounts of the alkaloids into brain and cerebrospinal fluid could not be demonstrated. Studies of ergot metabolism in which a colorimetric assay was employed provided somewhat higher recoveries in several organs (223), and



than have been employed Ergonovine produces quite significant depression of vasomotor and respiratory centers in anesthetized animals, although it elicits an increase in pressure (347)

It has been reported (350) that hydrogenation reduces the ability of the ergot alkaloids to depress the respiratory center, but that it does not alter their vasomotor depressant action It is unfortunate that this report does not include or refer to data regarding the extent and significance of this differential effect Dihydroergotamine and dihydroergocornine produce less respiratory depression in anesthetized dogs than does ergotamine (211) However, all of the derived alkaloids, in the doses required to block responses to sympathetic nerve stimulation do produce marked respiratory depression in anesthetized cats (295)

Ergotamine produces a marked stimulation of somatic motor neurones (339), but the extent to which this action is shared by the dihydro alkaloids has not been determined Reports that dihydroergotamine is 6 to 8 times less emetic than ergotamine (348, 350) do not appear to have been substantiated The ratio has since been observed to be about 1:2 in puppies (305) and not greater than 1:2 in patients with migraine (398) Other dihydro derivatives also appear to have a strong emetic action in man (34, 36, 37, 121, 167), as little as 0.3 mgm of dihydroergocornine may produce vomiting (34, 36) A report of antagonism to apomorphine emesis in dogs by supra-emetic doses of dihydroergotamine is incomplete (224), but this antagonism appears to be due to brain-stem depression, ergotamine produces an exactly comparable effect (73) Ergotamine raises the electroshock seizure threshold in rabbits (162), an action which is obviously not due to adrenergic blockade because ergonovine has an equal effect

The ergot alkaloids rather specifically depress certain centers in the brain stem The effects of  $\text{CO}_2$  on blood pressure and respiration are readily inhibited, apparently by decreasing the responsiveness of medullary centers to direct stimulation by  $\text{CO}_2$  (235) Depression of the response to  $\text{CO}_2$  occurs with doses of ergotamine which do not inhibit chemoreceptor reflexes or prevent the vascular effects of epinephrine or splanchnic nerve stimulation Dihydroergotamine appears to be somewhat less effective than ergotamine in reducing medullary sensitivity to  $\text{CO}_2$  (110, 350) Other dihydro ergot alkaloids have not been tested for this action

Ergot alkaloids also inhibit vascular responses to carotid baroreceptor (110, 111, 112) and chemoreceptor (137, 252) reflexes in doses which do not significantly alter vascular responses to injected epinephrine or direct splanchnic nerve stimulation Dihydroergotamine appears to be about as effective as ergotamine in this respect, although it has been stated without the presentation of substantiating data, that dihydroergotamine and dihydroergocornine produce somewhat less depression of carotid sinus reflexes than does ergotamine (350) The complete dissociation of this inhibition of vasomotor reflexes on the basis of brain-stem depression from adrenergic blockade is emphasized by the fact that adrenergically inactive ergonovine is about one-half as potent as ergotamine in depressing responses to carotid sinus nerve stimulation (347)

Electrical recording from the splanchnic nerves has indicated that the flow of

convincing evidence against such an effect. Vasodilatation caused by a single injection of Priscol in the presence of an epinephrine perfusion (266) or infusion (276) is very transient.

Priscol inhibition of the pressor response to both epinephrine and electrical stimulation of the splanchnic nerves in cats has recently been studied quantitatively (71). It was found that distinctly higher doses of the blocking agent are required to inhibit responses to nerve activity than to block those to circulating epinephrine, a relationship also observed in dogs (185, 187). A similar differential was noted in the inhibition of salivary secretion. It has also been demonstrated that larger doses of Priscol are required to inhibit responses to pressor reflexes than to block pressor responses to epinephrine (187, 254). Epinephrine-induced contractions of the rabbit uterus *in vitro* and the pregnant dog uterus *in situ* are also readily inhibited and reversed (11).

Mydriasis in response to cervical sympathetic nerve stimulation is unaltered and that to injected epinephrine is only slightly reduced by Priscol (71, 187). Epinephrine-induced contraction of iris muscles *in vitro* is also resistant to Priscol blockade (140). In intact animals (140, 187) and man (151) mydriasis rather than miosis may be produced, an effect which has been attributed to inhibition of the response of the iris sphincter to normally prevalent cholinergic stimuli (140). A cholinergic blocking action is also implied in the reported inhibition of the vasodepressor response to stimulation of the peripheral end of the severed vagus (255). These observations are at variance with reports of potentiation by Priscol of cholinergic responses of other organs (145), and require confirmation.

The observation that Priscol transforms epinephrine vasodilatation in the femoral and mesenteric circulations to vasoconstriction (260) has not been repeated or adequately explained. Other workers (145) have noted the expected change in response to epinephrine from vasoconstriction to vasodilatation after treatment with Priscol. A more active congener of Priscol (see III, C) also fails to alter the response to epinephrine from dilator to constrictor in any vascular bed (263).

Orally administered Priscol effectively protects mice against epinephrine toxicity (240). This action probably depends upon both the adrenergic blocking and the direct vasodilating actions of Priscol. The drug also transforms epinephrine apnea to hyperpnea (185). The basis for this action is not clear, but it is not dependent on inhibition of the pressor response to epinephrine because it is evident after denervation of the baroreceptor areas. Priscol does not alter the hyperglycemic action of epinephrine and the drug itself produces an increase in blood glucose (187).

In contrast to the  $\beta$ -haloalkylamines, Priscol has a relatively short duration of action (70, 217, 295) and a limited effectiveness against responses to large doses of epinephrine (145, 295, 298). An "equilibrium" type blockade appears to be involved in the Priscol inhibition of responses to epinephrine (145, 295) and to a large series of other sympathomimetic amines (11). In this respect the imidazolines are comparable to the benzodioxane blocking agents rather than to the  $\beta$ -haloalkylamines.

demonstrated that chloroform or phosphorus liver damage, but not bilateral nephrectomy significantly slows destruction of the alkaloids. Inasmuch as the color test employed is specific for the 2-unsubstituted indol group of the lysergic acid nucleus, these results indicate that the nucleus itself is rapidly degraded in the body.

### III IMIDAZOLINES

The pharmacology of Priscol (2-benzyl-2-imidazoline) was first reported by Hartmann and Isler in 1939 (164) who found it to be the most active depressor agent in a large series of 2-substituted imidazolines. They did not, however, demonstrate adrenergic blockade and compared the actions of Priscol with those of histamine, a comparison probably suggested by the common imidazole radical. Meier and Müller (262) made similar observations, and also noted a number of parasympathomimetic actions. They reported that Priscol did not effectively antagonize epinephrine vasoconstriction in the perfused rabbit ear, or prevent the pressor response to epinephrine unless the two were administered simultaneously.

#### A *Adrenergic blocking action*

1 "*Excitatory*" responses The first recognition of the adrenergic blocking action of Priscol appears in the work of Schnetz and Fluch (363), who observed that it blocks the vasoconstrictor action of epinephrine in the Lâwen-Trendelenburg frog-leg perfusion preparation. Meyer (266) and Meier and Meyer (261) later observed that Priscol produces marked vasodilatation, not blocked by atropine, in the isolated rabbit limb perfused with epinephrine, and Hermann and coworkers (185) noted that Priscol inhibits and occasionally even reverses the pressor response to stimulation of the peripheral stump of the severed splanchnic nerves.

The adrenergic blocking action of Priscol has been studied on many test objects. Inhibition of epinephrine vasoconstriction in discrete vascular beds or in intact animals has been demonstrated by numerous investigators (11, 71, 145, 164, 226, 261, 276, 363), but in many reports on the depressor and vasodilator actions no real attempt was made to distinguish between the effects of adrenergic blockade and direct vasodilatation. The locus of Priscol vasodilatation, both direct and secondary to adrenergic blockade, is largely peripheral. Vasodilatation occurs after local application (140, 164, 262, 363, 412), iontophoresis (412) and intra-arterial injection or perfusion (11, 262, 266). It has also been suggested (262) that a medullo-spinal vasodilating mechanism is involved, but convincing proof of this contention has not been presented. An important direct action of Priscol on peripheral vessels (see also III, B-1, B-3, VII-A) is suggested by the fact that it causes vasodilatation in some previously sympathectomized limbs (151, 236), induces coronary dilatation (11) and evokes peripheral vasodilatation in doses which do not inhibit responses to even small amounts of injected epinephrine (184). Failure to demonstrate direct vasodilatation in perfused limbs in which the vessels are probably already maximally dilated (266) is not

methylimidazoline (123)) or sympathomimetic (Privine, 2-naphthylmethylimidazoline (82) and Otrivine, 2-phenylaminomethylimidazoline (309)) Under appropriate conditions large doses of Privine may exhibit adrenergic blocking activity by reversing the pressor response to epinephrine (105, 260) N-methyl-substitution of Priscol endows it with pressor properties (145, 164) which are secondary to a nicotinic action on sympathetic ganglia (145) Actions of Priscol other than adrenergic blockade are particularly well illustrated in the observations of Ahlquist and coworkers (11)

*1 Sympathomimetic effects* Several properties of Priscol have been described as sympathomimetic This agent elicits a tachycardia and increased stroke volume in intact dogs (25, 298) and tachycardia, coronary dilatation and increased cardiac output in isolated mammalian hearts (11, 23, 145, 333) Priscol-induced cardiac stimulation has also been reported with therapeutic doses in man (11, 184, 276), although other workers (151) have observed only minimal tachycardia with tolerated doses Tachycardia is a prominent sign of Priscol toxicity in man (272) Reasons for the failure of earlier workers to demonstrate significant coronary dilatation and myocardial stimulation in isolated mammalian hearts (262) and heart-lung preparations (166) are not apparent, although recent observations (145) indicate that some species differences may have been involved Stimulation of the isolated guinea pig heart is such a sensitive test for Priscol that it has been employed as an assay in experiments designed to determine the fate of this agent in the body (333) Priscol is less effective in stimulating the frog heart and higher concentrations depress it (25, 164, 276, 295)

In dogs the net cardiovascular response to Priscol may actually be an increase in systemic arterial pressure (11, 185, 254, 262), probably because of dominance of an increased cardiac output (frequently more than doubled by moderate doses) over the peripheral vasodilatation (11) Therapeutic doses of Priscol also have been noted to cause an alarming hypertension in man (28), probably on the same basis Increased peripheral resistance is probably not a significant factor in the hypertensive action of Priscol in the dog, although it may occur in the rabbit (11) (see III, B-3)

Priscol potentiates the myocardial stimulation and coronary dilatation caused by epinephrine (11) It may also produce transient relaxation of the gastrointestinal musculature (11) Whether the pilomotor response to Priscol which is prominent in both animals (262, 276) and man (52, 151, 184, 427), is direct or reflex in nature cannot be stated on the basis of the published data

Evidence for the sympathomimetic nature of the above enumerated effects of Priscol is largely indirect and negative In general, inclusion in this category has been based upon the similarity of the responses to those evoked by epinephrine, and upon the failure of atropine to block them

*2 Parasympathomimetic effects* Priscol may be compared chemically to pilocarpine on the basis of the imidazole grouping, and this radical may be important for its parasympathomimetic properties Parasympathomimetic actions of Priscol include cardiac slowing (observed only in rabbits (11)) and stimulation

2 *Cardiac responses* The effects of epinephrine on the mammalian heart either isolated or *in situ* are not inhibited by Priscol (11, 145, 259, 298) No explanation for a report of reversal of the cardiac response to stellate ganglion stimulation (313) is available, but the observation probably depends on technical factors (see I, A-4) because nitroglycerin and papaverine also produced apparent adrenergic blockade in these experiments Priscol potentiates the myocardial stimulation and coronary dilatation evoked by epinephrine (11) In contrast to members of several other series of adrenergic blocking agents Priscol does not effectively antagonize the response of the frog heart to epinephrine (276, 295).

Although Priscol does not inhibit epinephrine-induced tachycardia in dogs, it does provide marked protection against cyclopropane-epinephrine cardiac arrhythmias (298) The protection is essentially the same as that observed with Dibenamine when small challenge doses of epinephrine are employed, but larger doses of epinephrine overcome the Priscol protection whereas the Dibenamine protection remains essentially complete Larger doses of Priscol are required to protect against epinephrine-induced cardiac arrhythmias than to inhibit the epinephrine pressor response

3 *"Inhibitory" responses* Inhibitory responses (non-pregnant cat uterus, dog, cat, guinea pig and rat intestine) to epinephrine are not blocked by Priscol (11, 187, 276) The agent appears to inhibit slightly the epinephrine-induced relaxation of some rabbit ileum preparations, and of the cat intestine *in vivo* (145) and *in vitro* (276) However, on the rabbit ileum this antagonism is very irregular and no greater than that exerted by ephedrine and Neo-Synephrine Inhibition of epinephrine-induced relaxation of the cat intestine *in vitro* requires extremely high doses Responses to vasodepressor amines are not altered by Priscol (11, 295)

4 *Specificity* Priscol does not inhibit the pressor response to posterior pituitary, renin or angiotonin (11, 71) Histamine vasoconstriction in the perfused rabbit limb is not altered by doses of Priscol which inhibit the response to epinephrine, and the vasoconstrictor effect of  $\text{BaCl}_2$  may actually be enhanced (266) However, high concentrations of Priscol can prevent histamine vasoconstriction in the perfused rabbit ear (145) This action is not surprising when one considers the close relationship of Priscol to the active antihistaminic Antistine

### B Actions other than adrenergic blockade

In addition to their adrenergic blocking action, the imidazoline blocking agents appear to have some direct effect upon almost every organ in the body These effects are highly varied, they have been assigned to categories such as "sympathomimetic" and "parasympathomimetic" by various investigators primarily as a matter of convenience Such a classification should not carry implications regarding the intimate mode of action involved

The lack of specificity of the imidazoline adrenergic blocking agents is not surprising since only slight changes in structure produce compounds with actions which are predominately antihistaminic (Antistine, 2-(N-benzylanilino)methyl-

renergic blockade produced appears to be quite similar to that of Priscol in that it is not effective against large doses of epinephrine and its duration is relatively short (295). Metabolic responses to epinephrine are not blocked by #7337, and limited studies have not demonstrated antagonism of inhibitory responses.

Unfortunately, insofar as #7337 has been studied, it appears to possess only slightly greater specificity than Priscol. The direct depressor effect is only slightly decreased and appears with doses which produce little alteration of carotid sinus pressor reflexes. Cardiac stimulation in the dog is only slightly reduced. Stimulation of the guinea-pig ileum *in vitro* is much less than observed with comparable doses of Priscol (295), but the intestine *in situ* is stimulated and diarrhea is prominent after the administration of relatively small doses to unanesthetized animals (263).

#### IV BENZODIOXANES

The past decade has produced few fundamental changes in our understanding of the adrenergic blocking activity of members of the benzodioxane series (see 47, 407). Coumarane derivatives (see 18, 47, 49) have been studied much less thoroughly and have received little recent attention. Most of their properties are similar to those of the benzodioxanes and they will not be discussed separately.

##### A Adrenergic blocking action

1 "Excitatory" responses Since the first report of the adrenergic blocking action of the benzodioxanes (118), numerous experiments have demonstrated blockade of excitatory adrenergic responses of the general circulation, renal vascular bed, nictitating membrane, iris and certain uteri. Some members of the series are effective primarily against responses to circulating sympathomimetic agents (933F type) while others are effective against responses to both circulating mediators and sympathetic nerve activity (883F type) (see 19). However, all combinations of intensities of these two properties are present within the series (47). Responses to circulating mediator (including sympathin (17, 192, 238) and norepinephrine (264)) are always blocked more readily than responses to sympathetic nerve stimulation, even in the case of the salivary glands where nerve endings are supposed to be extracellular (275). Even 933F produces significant blockade of responses to sympathetic nerve stimulation (17, 18, 338) and to carotid cardiovascular reflexes (252, 407). Conversely, 883F is quite ineffective against the excitatory responses of some organs (e.g., the iris) to sympathetic nerve stimulation (369). Low concentrations of 933F potentiate the effects of epinephrine and sympathetic nerve stimulation on the perfused rabbit ear (204). Here also, higher concentrations are required to alter the response to nerve stimulation than that to injected epinephrine. Efforts to localize the action of certain benzodioxanes and other agents at the cell surface on the basis of quantitative differences in effectiveness against responses to circulating mediator and sympathetic nerve activity lack convincing support (see 275, 292).

of the intact gastrointestinal tract (11, 145, 184, 276, 428) The responses are blocked by atropine, a property which is usually considered to be evidence for their cholinergic nature A miotic effect (71) and stimulation of salivary (71, 187), pancreatic (187) and respiratory tract (298) secretion have also been reported, but the influence of atropine on these effects has not been studied An overdose of Priscol was noted to produce profuse sweating in man (272),

Priscol evokes submaxillary secretion by a direct action in the gland cells because it is observed even after denervation (187) Priscol potentiates the responses of a number of organs to acetylcholine (145) perhaps through an inhibition of cholinesterase (266, 361)

*3 Histamine-like effects* Certain responses to Priscol have also been attributed to histamine-like properties These include vasoconstriction in the rabbit (11, 276), stimulation of the isolated gut not blocked by atropine (11, 266), stimulation of the uteri of dogs, cats, guinea pigs and rabbits *in vivo* and *in vitro* (11, 276) and stimulation of the intestine and nictitating membrane of the intact cat (145) Priscol potentiates the effects of histamine on several of these structures Peripheral vasodilatation is prominent (11, 71, 164, 261, 262), is not blocked by atropine and appears with doses of the drug which do not inhibit the pressor response to small amounts of epinephrine (184) Some workers have classified this action as sympathomimetic (11) Priscol-induced vasoconstriction in the dog spleen and kidney (185, 187) may belong in this category, but other workers have failed to confirm these effects (145) No attempt has yet been made to employ antihistaminic drugs in analyzing the above "histamine-like" actions of Priscol

Priscol stimulates gastric secretion of both acid and pepsin in man and animals (52, 145, 364, 395) It is only slightly less effective than histamine, and has been substituted for the latter as a test for gastric secretion in man with apparent success (52, 278, 364, 395)

*4 Miscellaneous effects* Priscol inhibits oxidative metabolism in kidney slices and is destroyed by liver slices (23, 333) The pharmacological significance of these observations is not clear Priscol is also an active inhibitor of monoamine and diamine oxidases (361), but this property appears to be unrelated to adrenergic blockade The sympathomimetic imidazolines Privine and Otrivine produce a similar inhibition

*C Other imidazolines with adrenergic blocking activity* Although many congeners of Priscol have been synthesized, the adrenergic blocking properties of only 2-(N, p-tolyl-N-(m'oxy-phenyl)-aminomethyl)-imidazoline (#7337) has received detailed attention (263) This agent is more potent than Priscol in blocking the pressor response to epinephrine and the salivary secretion induced by epinephrine and cervical sympathetic nerve stimulation It is also a very effective antagonist of epinephrine-induced contraction of the guinea pig seminal vesicle *in vitro* In contrast to Priscol, #7337 blocks mydriasis in response to cervical sympathetic stimulation However, large doses are required and this effect may only be a reflection of quantitative differences in potency The ad-

renergic blockade produced appears to be quite similar to that of Priscol in that it is not effective against large doses of epinephrine and its duration is relatively short (295). Metabolic responses to epinephrine are not blocked by #7337, and limited studies have not demonstrated antagonism of inhibitory responses.

Unfortunately, insofar as #7337 has been studied, it appears to possess only slightly greater specificity than Priscol. The direct depressor effect is only slightly decreased and appears with doses which produce little alteration of carotid sinus pressor reflexes. Cardiac stimulation in the dog is only slightly reduced. Stimulation of the guinea-pig ileum *in vitro* is much less than observed with comparable doses of Priscol (295), but the intestine *in situ* is stimulated and diarrhea is prominent after the administration of relatively small doses to unanesthetized animals (263).

#### IV BENZODIOXANES

The past decade has produced few fundamental changes in our understanding of the adrenergic blocking activity of members of the benzodioxane series (see 47, 407). Coumarane derivatives (see 18, 47, 49) have been studied much less thoroughly and have received little recent attention. Most of their properties are similar to those of the benzodioxanes and they will not be discussed separately.

##### A Adrenergic blocking action

1 "Excitatory" responses Since the first report of the adrenergic blocking action of the benzodioxanes (118, numerous experiments have demonstrated blockade of excitatory adrenergic responses of the general circulation, renal vascular bed, nictitating membrane, iris and certain uteri. Some members of the series are effective primarily against responses to circulating sympathomimetic agents (933F type) while others are effective against responses to both circulating mediators and sympathetic nerve activity (883F type) (see 19). However, all combinations of intensities of these two properties are present within the series (47). Responses to circulating mediator (including sympathin (17, 192, 238) and norepinephrine (264)) are always blocked more readily than responses to sympathetic nerve stimulation, even in the case of the salivary glands where nerve endings are supposed to be extracellular (275). Even 933F produces significant blockade of responses to sympathetic nerve stimulation (17, 18, 338) and to carotid cardiovascular reflexes (252, 407). Conversely, 883F is quite ineffective against the excitatory responses of some organs (*e.g.*, the iris) to sympathetic nerve stimulation (369). Low concentrations of 933F potentiate the effects of epinephrine and sympathetic nerve stimulation on the perfused rabbit ear (204). Here also, higher concentrations are required to alter the response to nerve stimulation than that to injected epinephrine. Efforts to localize the action of certain benzodioxanes and other agents at the cell surface on the basis of quantitative differences in effectiveness against responses to circulating mediator and sympathetic nerve activity lack convincing support (see 275, 292).



The adrenergic blockade produced by the benzodioxanes is rather weak, in that it is readily overcome by large doses of epinephrine (295) and rapidly disappears in the presence of a continuous epinephrine infusion (186). The *l*-form of 883F is about six times as potent in producing adrenergic blockade, and twice as toxic as the *d*-isomer (46). Other members of the series have not been resolved.

It has recently been reported (240) that orally administered 883F or 933F fails to protect against epinephrine toxicity in mice, and 933F was also found to be ineffective when administered in large doses subcutaneously one hour prior to the epinephrine. Inasmuch as 933F provides definite protection in rats when a shorter time interval is employed (312), and in mice (see 47), the only immediate explanation for the reported negative results appears to be that the animals were not tested soon enough after administration to detect the rather transient blockade produced by these agents. Unfortunately, no attempt has been made to determine the time of peak effect.

Stimulation of the central nervous system by sympathomimetic amines is not blocked by 933F or 883F (78).

Responses of the isolated frog heart to epinephrine and sympathin are inhibited or reversed by the benzodioxanes (204, 238, 367, 368), although some workers (194) have failed to confirm this action. Changes in the composition of the perfusion medium may greatly modify the ability of the ergot alkaloids to block adrenergic responses of the frog heart (see II, C-2), and it is possible that a similar factor may explain the divergent results obtained with the benzodioxanes. The effects of epinephrine on the mammalian myocardium are not specifically inhibited (194, 253, 407).

Benzodioxane derivatives have been reported in common with ergotamine and certain phenoxyethylamines, to prolong significantly the bleeding time, presumably by inhibiting reflex sympathetic vasoconstriction (95). This action is not shared by related compounds (such as 933F) which are relatively ineffective in blocking responses to sympathetic nerve activity. The fact that benzodioxanes fail to alter the hemostatic effect of injected epinephrine (94) appears to be good evidence that an altered conversion of epinephrine or sympathin to adrenochrome is not involved in the prolongation of bleeding time reported above. Although 933F has been reported to promote the inactivation of epinephrine *in vitro* (275), there is no evidence that this adrenolytic action is involved in the production of adrenergic blockade. Yohimbine and phenoxyethylamines (which produce an adrenergic blockade very similar to that of the benzodioxanes) have been shown not to alter the disappearance of epinephrine from the blood stream (63).

2 "Inhibitory" responses. Studies of the effect of benzodioxanes on inhibitory responses to adrenergic stimuli have not yielded conclusive results. Epinephrine-induced coronary artery dilatation is not altered by 933F (213), an apparent reduction in epinephrine-induced coronary artery dilatation by 883F (89) may be largely a passive effect of inhibition of the pressor response to epinephrine, although direct coronary artery constriction by 883F (see IV, B-1)

cannot be ruled out as a contributing factor. It has been observed that 933F inhibits epinephrine-induced relaxation of rat and rabbit intestine only in concentrations which also inhibit the myotropic action of  $\text{BaCl}_2$  or directly affect the tonus of the test object (136, 180, 274). Epinephrine-induced relaxation of the guinea pig intestine may be somewhat more readily inhibited (178, 180), but in the required concentrations 933F itself induces a very marked relaxation of the test object.

The non-pregnant cat uterus provides a somewhat more reliable test object than intestine upon which blockade of adrenergic inhibitory responses may be studied. The benzodioxanes have never been shown to produce more than minor alterations in the response of this organ to epinephrine (83, 341), and some studies have failed to demonstrate any blockade of the epinephrine inhibition (18, 84). Indeed, at times 933F may actually potentiate the epinephrine-induced relaxation. The benzodioxanes act directly to stimulate the uterus of several species (18, 83), a property which seriously complicates the interpretation of subsequent responses to epinephrine. The evidence for a specific blockade of adrenergic inhibitory responses by the benzodioxanes is not conclusive. Variations in experimental conditions as well as in the species and organs employed may be involved in the discrepancies reported. A comprehensive, well controlled study of the entire problem is needed.

*3 Specificity* Benzodioxanes have been reported not to block pressor responses to nicotine, KCl,  $\text{BaCl}_2$ , posterior pituitary and  $\beta$ -tetrahydronaphthylamine (see 407). In addition, the pressor response of both normal and renal hypertensive dogs to renin is unaltered by 933F (212). Observations indicating a reduced response of the nictitating membrane of the cat to acetylcholine, KCl, and  $\text{CaCl}_2$  after the administration of 933F (340) have not been explained or repeated.

### *B Actions other than adrenergic blockade*

*1 Smooth muscle* The benzodioxanes directly stimulate many different types of smooth muscle including those of uterus, gut, bronchi and nictitating membrane (18, 47, 83). Coronary vessels are strongly constricted (89, 213), and it is impossible to find a theoretical basis for the trial of 883F in the therapy of angina pectoris (77). The agents also exert a potent direct constrictor action on peripheral vessels which is responsible for the pressor effect in dogs after phthying (206) or complete spinal anesthesia (208).

*2 Cardiac muscle* Effects of 883F and other benzodioxanes on the mammalian heart appear to be best explained on the basis of a direct myocardial depression (207, 382), indeed, 933F has been found to be approximately 3 times as active a myocardial depressant as quinidine (92). Isolated cat, rat and guinea pig hearts are directly depressed by lower concentrations of 933F than are required to alter the response to epinephrine (253). Specific blockade of adrenergic cardiac acceleration by these agents in mammals is very limited or absent. Doses of 883F or 933F which reverse the pressor effects of epinephrine in anesthetized animals do not alter epinephrine-induced tachycardia (407).

The adrenergic blockade produced by the benzodioxanes is rather weak, in that it is readily overcome by large doses of epinephrine (295) and rapidly disappears in the presence of a continuous epinephrine infusion (186). The *l*-form of 883F is about six times as potent in producing adrenergic blockade, and twice as toxic as the *d*-isomer (46). Other members of the series have not been resolved.

It has recently been reported (240) that orally administered 883F or 933F fails to protect against epinephrine toxicity in mice, and 933F was also found to be ineffective when administered in large doses subcutaneously one hour prior to the epinephrine. Inasmuch as 933F provides definite protection in rats when a shorter time interval is employed (312), and in mice (see 47), the only immediate explanation for the reported negative results appears to be that the animals were not tested soon enough after administration to detect the rather transient blockade produced by these agents. Unfortunately, no attempt has been made to determine the time of peak effect.

Stimulation of the central nervous system by sympathomimetic amines is not blocked by 933F or 883F (78).

Responses of the isolated frog heart to epinephrine and sympathin are inhibited or reversed by the benzodioxanes (204, 238, 367, 368), although some workers (194) have failed to confirm this action. Changes in the composition of the perfusion medium may greatly modify the ability of the ergot alkaloids to block adrenergic responses of the frog heart (see II, C-2), and it is possible that a similar factor may explain the divergent results obtained with the benzodioxanes. The effects of epinephrine on the mammalian myocardium are not specifically inhibited (194, 253, 407).

Benzodioxane derivatives have been reported in common with ergotamine and certain phenoxyethylamines, to prolong significantly the bleeding time, presumably by inhibiting reflex sympathetic vasoconstriction (95). This action is not shared by related compounds (such as 933F) which are relatively ineffective in blocking responses to sympathetic nerve activity. The fact that benzodioxanes fail to alter the hemostatic effect of injected epinephrine (94) appears to be good evidence that an altered conversion of epinephrine or sympathin to adrenochrome is not involved in the prolongation of bleeding time reported above. Although 933F has been reported to promote the inactivation of epinephrine *in vitro* (275), there is no evidence that this adrenolytic action is involved in the production of adrenergic blockade. Yohimbine and phenoxyethylamines (which produce an adrenergic blockade very similar to that of the benzodioxanes) have been shown not to alter the disappearance of epinephrine from the blood stream (63).

2 "Inhibitory" responses. Studies of the effect of benzodioxanes on inhibitory responses to adrenergic stimuli have not yielded conclusive results. Epinephrine-induced coronary artery dilatation is not altered by 933F (213), an apparent reduction in epinephrine-induced coronary artery dilatation by 883F (89) may be largely a passive effect of inhibition of the pressor response to epinephrine, although direct coronary artery constriction by 883F (see IV, B-1).

properties has been reported, and large doses of 933F may also produce anesthesia (160)

When applied locally the benzodioxanes also block ganglionic transmission (17) Whether this is a true "nicotinic" ganglionic blockade or merely a local anesthetic effect (49) is not apparent from the published results

It is unfortunate that the central nervous system actions of the benzodioxanes have not been studied by more direct neurophysiological techniques At the present time it is impossible to evaluate clearly the relative contributions of peripheral adrenergic blockade and central nervous system stimulation and depression to the over-all pharmacological properties of members of this series

## V YOHIMBINE

Despite the fact that its adrenergic blocking action has been known since 1925 (314), yohimbine has had only limited use as a laboratory tool and has not been employed therapeutically as a blocking agent

### A Adrenergic blocking action

1 "Excitatory" responses Yohimbine and ethyl-yohimbine block certain responses to both circulating epinephrine and sympathetic nerve activity, but sympathetic blockade is produced only by doses two to five times greater than those required to prevent responses to epinephrine (24, 430) Failure of some investigators to demonstrate blockade of the pressor effects of splanchnic nerve stimulation by yohimbine (225) is probably due to the use of inadequate doses Very low concentrations of yohimbine sensitize the perfused rabbit's ear to epinephrine (204), an action shared by several other adrenergic blocking agents In the cat, pressor responses and salivary secretion are more readily inhibited than ocular smooth muscle responses (430), a fact which may largely explain the lack of "sympatholytic" activity reported on the basis of tests on the mictating membrane and iris (17, 369) Yohimbine adrenergic blockade has also been demonstrated on ocular smooth muscles, arterial strips and rabbit uteri *in vitro* (69, 198, 355) Orally administered yohimbine provides significant, but not marked, protection against the lethal effects of epinephrine in mice (240) Greater protection would probably be provided by parenteral injection Yohimbine blockade of cardiovascular responses to epinephrine is not altered by any of a considerable number of anesthetic agents (198, 225)

Yohimbine readily reverses the pressor response to acetylcholine in anesthetized, atropinized animals, but only reduces the response to nicotine (366) This difference in action is similar to that noted with Dibenzamine, and the same possible explanations apply (see I, A-2) The glycemic response to epinephrine is inhibited (161)

Yohimbine blockade of the responses of arterial strips to epinephrine *in vitro* is much less complete in the presence of cocaine (69) The adrenergic blocking action of the benzodioxanes is also antagonized by cocaine (19), but that of the  $\beta$ -haloalkylamines is not significantly altered by this agent (295) The difference is probably due to the fact that yohimbine and the benzodioxanes produce an

The non-specificity of the cardiac effects of these agents on the mammalian heart is emphasized by the fact that electrically-induced fibrillation and  $\text{BaCl}_2$ -induced ectopic rhythms are inhibited as readily by 933F as epinephrine arrhythmias (96), and the fact that the response to vagal stimulation is reduced almost parallel to that to accelerator nerve stimulation (407)

Chloroform-epinephrine ventricular fibrillation is prevented by 933F and 883F (370) Inhibition of the pressor response to epinephrine has been advanced as the basis for this protection, although some of the published records indicate that the pressure may rise as much in the presence of 883F as in unprotected animals prior to the onset of arrhythmias Corynanthine also affords marked protection despite epinephrine-induced pressure rises of as much as 140 mm Hg (372) Pressure is probably a factor in the induction of cardiac arrhythmias, but the absolute pressure rather than the magnitude of the rise appears to be the primary sensitizing factor (see I, A-5) Fibrillation induced by digitals overdosage in cats is not inhibited by 883F or 933F (104)

*3 Central nervous system* Stimulation of the central nervous system by the benzodioxanes appears to affect particularly the lower brain stem At least among piperidine derivatives of benzodioxane, this effect is roughly parallel to adrenergic blocking activity (47) Central nervous system stimulation is probably involved in the production of hypertension in unanesthetized animals (29, 47, 172) and man (143)

Administration of the benzodioxanes to anesthetized dogs may produce a vagus-mediated bradycardia even in the presence of a systemic hypotension (172, 407) This response is followed by a secondary cardiac acceleration which is prevented by ganglionic blockade or cardiac denervation but not by removal of the adrenal glands (172, 407) The hyperglycemic response to the administration of the benzodioxanes (47, 177) is also probably due to a central action mediated through the sympatho-adrenal system, although the same compounds have been reported to inhibit the hyperglycemic action of epinephrine (33, 47, contrast 177) Antidiuresis, presumably induced by hypothalamic stimulation, has also been noted (434)

Although the reported central nervous system effects of the more commonly employed members of the benzodioxane series are primarily excitatory, elements of brain-stem depression are also present The hypotension induced by these agents in anesthetized animals is at least partially due to a depression of central vasomotor tone (206, 208, compare 407) Inhibition of responses to carotid reflexes, and to stimulation of the central stump of the severed vagus, by doses of 883F which do not inhibit responses to splanchnic nerve stimulation (407), also indicates brain-stem depression Although a local anesthetic action of the benzodioxanes on carotid receptors represents another possible mechanism of action (421), concentrations adequate to produce this effect (0.15%) are probably not attained in intact animals Other manifestations of central nervous system depression are analgesia, suppression of vomiting due to digitals or apomorphine, and prolongation of the action of other central nervous system depressants (49) An adrenergic-blocking benzodioxane derivative with predominately anesthetic

the observation (24) As in the case of observations on the blockade of adrenergic stimulation of the frog heart by the ergot alkaloids (see II, C-2), differences in technic may account for these divergent observations It has also been reported that yohimbine may depress the frog heart in lower concentrations than those required to inhibit the response to epinephrine (198), an observation which must be kept in mind in an evaluation of the reported inhibition of adrenergic cardiac responses

3 *"Inhibitory" responses* Yohimbine has been reported to antagonize epinephrine relaxation of rabbit and cat intestine (198, 431), but very high concentrations (1 1000) are required to block responses of the latter Rat intestine is relaxed by yohimbine (198) The limited number of observations make any definite conclusion regarding the blockade of adrenergic inhibitory responses by yohimbine impossible In view of the limited specificity of enteric organs as test objects (see II, C-3), it must be concluded that specific blockade of adrenergic inhibitory responses by yohimbine has not been established

4 *Specificity* Ethyl-yohimbine, even in large doses, does not alter the vascular or ocular smooth muscle responses to angiotonin and posterior pituitary (429), nor does yohimbine suppress the pressor effect of KCl in adrenalectomized dogs (179) The actions of histamine, barium chloride and nitrite on arterial strips *in vitro* are also unaffected (69) The significance of a reported reduction by yohimbine of responses of the cat mictitating membrane to KCl, BaCl<sub>2</sub> and acetylcholine (340) is not clear

### *B Actions other than adrenergic blockade*

1 *Central nervous system* Yohimbine has several well-established effects on the central nervous system Anti-diuresis is prominent (130, 437), and appears to be due to the release of posterior pituitary hormone in response to hypothalamic stimulation It does not occur after section of the hypophyseal stalk (130) Corynanthine and yohimbine also induce melanophore expansion in intact but not in hypophysectomized frogs (393) This effect appears not to involve nervous mediation from the hypothalamus because it is not prevented by the application of local anesthetics to the hypophyseal stalk (371) However, proof that local anesthetics adequately penetrate the hypophyseal stalk after topical application would make this interpretation more certain

Yohimbine inhibits epinephrine- (141) and ergotamine-induced (176) apnea in anesthetized dogs, despite the fact that the drug itself can produce apnea However, the reports provide little or no evidence regarding the significance of these phenomena, or of the failure of rabbits to respond in a comparable manner (175) Although nicotine apnea is apparently unaffected (141) the above alterations in respiration are probably manifestations of non-specific stimulation of the central nervous system by yohimbine There is no reason to believe that they are related to the protection against anoxia which this agent is reported to provide (106)

Brain-stem depression also appears to be involved in the anti-emetic action of yohimbine (72) Toxic doses of yohimbine first stimulate and then depress

"equilibrium" blockade which may be overcome by increased doses of epinephrine (295, 327), while the  $\beta$ -haloalkylamines produce a "non-equilibrium" blockade (see I, C-2). Although the exact mechanism of the sympathomimetic action of cocaine is unknown, the drug potentiates many responses to epinephrine. This potentiation provides an adequate basis for explaining the above effects of cocaine on "equilibrium" blockade.

The fact that yohimbine reversal of the nicotinic pressor response to acetylcholine is enhanced rather than depressed by cocaine (366) may be reconciled with the observations discussed in the preceding paragraph on the basis that both excitatory and inhibitory effects of epinephrine are potentiated by cocaine and that inhibitory factors play a much more important role in responses of the intact vascular system than in responses of arterial strips. Yohimbine actually slightly potentiates responses to vasodepressor amines (168).

Yohimbine has been shown to suppress carotid cardiovascular reflexes in animals (24, 252, 403). In one of the few reported administrations of yohimbine to man (62), the agent significantly reduced orthostatic vasoconstriction. Central actions, such as are prominent in the case of ergot preparations (see II, D-2), may be involved in the depression of vasomotor reflexes by yohimbine. The pressor effects of carotid occlusion and of stimulation of the central end of the severed vagus nerve are inhibited by smaller doses than are required to block the effects of splanchnic stimulation (24). However, other observations (403) indicate that ordinarily effective doses of yohimbine do not inhibit carotid sinus initiated vasoconstriction when the drug is in contact only with the central nervous system and is excluded from both the sinus and the reacting limb. The possibility that yohimbine acts to block afferent impulses from the carotid area on the basis of its known local anesthetic action (317) must therefore be considered. Experiments involving perfusion of the vascularly isolated carotid sinus have demonstrated a local action by concentrations of yohimbine which may be attained *in vivo* (402). It is possible that adrenergic blockade, inhibition of central reflex pathways and local anesthesia of carotid receptors are all involved in the inhibition of vasomotor reflexes by yohimbine. A critical re-examination of this problem might be most rewarding.

*2 Cardiac responses* In common with other adrenergic blocking agents, yohimbine does not inhibit the chronotropic and inotropic effects of epinephrine on the mammalian heart (315). It has been reported that the rabbit heart is a qualitative exception to this general resistance to blockade (45), both in its response to yohimbine and that to several other blocking agents. However, more data on possible complicating experimental factors (see I, A-4) would seem desirable before this is accepted as a major exception to the generalization that chronotropic and inotropic responses of the mammalian myocardium are resistant to specific adrenergic blockade.

Yohimbine and corynanthine (an isomer of yohimbine) provide significant protection against chloroform-epinephrine ventricular fibrillation (370, 372). Yohimbine has also been reported to reduce but not to reverse the response of the frog heart to epinephrine (204), but other workers have failed to confirm

## VI MISCELLANEOUS ADRENERGIC BLOCKING AGENTS

In addition to the various groups of agents discussed above, a large number of miscellaneous compounds have been reported to exhibit adrenergic blockade. Few of these agents have been adequately studied with regard to the specificity of the blockade produced, and some of the published reports seemingly substantiate the dictum that "enough of anything will block anything." Only rarely have the compounds been compared quantitatively with more thoroughly studied agents, and therefore, an accurate evaluation of many of the substances to be discussed below is almost impossible.

*A Natural products*

A variety of compounds occurring in nature have been reported to possess adrenergic blocking activity. The pharmacological properties of the alstonia alkaloids (215) and purified alstonine (410), obtained from the bark of Australian trees of the genus *Alstonia*, very closely resemble those of quinine. Like quinine, they have very weak adrenergic blocking activity. Hydrocinchonidine appears to be the most active of the "quinine-like" alkaloids (323), but it is still far from being a potent adrenergic blocking agent. A quaternary derivative of hydrocinchonidine (329) is active although a similar derivative of yohimbine is inactive (328). Extracts of *Galium aparine* (93) and several species of *Rauwolfia* (see 316, 324, 325) have been reported to possess detectable adrenergic blocking activity, but their properties do not appear to be remarkable. It is possible that the active agents in extracts of *Rauwolfia* are closely related chemically to yohimbine.

Bulbocapnine (318) and boldine (320) are said to differ significantly from other adrenergic blocking agents in their ability to block inhibitory vascular responses to adrenergic stimuli as readily as excitatory responses. However, studies employing N-ethyl-norepinephrine (319) have demonstrated that inhibitory cardiovascular responses are largely unaffected by bulbocapnine. No proof of the specificity of the adrenergic blocking action of these compounds has been presented, and the published observations are most readily explained by the assumption that they are simply weak adrenergic blocking agents with a high non-specific toxicity for smooth muscle.

*B Synthetic compounds*

The first demonstration of adrenergic blocking activity by synthetic agents was that of Loewe in 1927 (243). He observed that a number of polyphenol-ethylamines inhibited and reversed the vasopressor and rabbit uterus responses to epinephrine. These agents received little additional attention, but this early work did much to stimulate the search for adrenergic blocking activity in synthetic compounds.

*1 Phenoxylethylamines* Interest in the phenoxylethylamine adrenergic blocking agents has been revived by reports of Lévy and coworkers on a series of primary and secondary phenoxylethylamines (57, 59, 210, 219, 220, 221, 234). Phenoxylethyl compounds first attracted attention about two decades ago because



respiration (433) Because of the subcortical central nervous system effects of yohimbine (and also of the benzodioxanes, see IV, B-3), the reported prevention of decerebrate rigidity by yohimbine and 933F (268) provides an inadequate basis for the conclusion that an "epinephrine-like" substance is involved in this phenomenon

It has been claimed that yohimbine causes prolonged estrus or pseudo-pregnancy in intact adult female rats but not in hypophysectomized or castrate animals (131), however, other work failed to confirm these results (391) It is clear that yohimbine exerts no endocrine effect in immature or castrate animals, but the claim that yohimbine activates the anterior pituitary has not been established The ejaculation elicited by yohimbine in mice treated with Pernoston (244) is inhibited by high section of the spinal cord (245) Present evidence provides no endocrine basis for the "aphrodisiac" effects of yohimbine and corynanthine The observed responses appear to depend entirely upon circulatory changes (322) and central nervous system stimulation

*2 Miscellaneous actions* Yohimbine hastens muscle fatigue and reduces skeletal muscle sensitivity to acetylcholine (401), but no evidence has been adduced to indicate that these effects involve adrenergic blockade, as in the case of Dibenamine inhibition of the anti-curare effect of epinephrine (see I, A-3) Yohimbine also produces mydriasis in mice by inhibiting cholinergic pupillary constriction (44) Both of these effects are probably related to the local anesthetic property of the agent

It appears that no physiological significance can be attached to the potentiation of phenol sulfur esterase activity by very high concentrations (5%) of yohimbine and ergotamine (399), or the inhibition by yohimbine, corynanthine and 933F of the potentiation of acetylcholine synthesis from choline by adrenochrome and cocarboxylase (267)

### C *Yohimbine congeners*

Except for ethyl-yohimbine, which has attracted attention because of its low toxicity (433), derivatives of yohimbine have received only superficial study Although adequate comparative data are not available, the ethyl, allyl, butyl, phenyl, diethylamine, ethylene glycol and diethylene glycol derivatives of yohimbine appear to have very similar pharmacological properties (69, 431, 433) Diacetylation, which presumably blocks the secondary amine and alcohol groupings, also has been reported not to alter significantly the activity of yohimbine and corynanthine (327), although others have reported that diacetylation increases by several fold the activity of corynanthine and certain other congeners (205, 307) The quaternary methyl-iodide of yohimbine is almost completely inactive (328) A number of other derivatives have been reported, but their properties do not appear to be unusual

Corynanthine, an isomer of yohimbine, possesses properties very similar to those of the latter Although it is less toxic (209, 354, 437) and appears to be a more active adrenergic blocking agent than yohimbine (44, 209, 354, contrast 205), this agent and its derivatives have been little studied

tion,  $\text{BaCl}_2$  and aconitine as readily as it does arrhythmias due to adrenergic stimuli (41, 51, 97). Indeed, careful measurements on the rabbit auricle *in vitro* (92) indicate that 1262F is 4 times as depressant as quinidine to the myocardium.

The many non-specific inhibitory effects of the phenoxyethylamines are probably related to a strong local anesthetic action (49, 50, 57, 210). Within the series, the most effective adrenergic blocking agents are among those with the strongest local anesthetic action (the secondary amines). The local anesthetic action of these agents has been directly implicated in their mydriatic action (44).

In general, phenoxyethylamines with a substitution in the 4, 3 or 2 position of the phenyl ring possess increasing adrenergic blocking activity in the order named. Di(2-methylphenoxyethyl)amine is the most active member of this series (210).

Substitution of the 2-methylphenoxyethyl radical for one or both of the benzyl groups of Dibenamine produces compounds with very high adrenergic blocking activity (288) (see I, D-3). In these compounds the presence of a  $\beta$ -chloroethyl moiety provides both a marked increase in potency and in duration of action. The high activity of members of both the  $\beta$ -haloalkylamine and the above primary and secondary amine series containing the 2-methylphenoxyethyl radical, implies some special role for this grouping. In view of the very different chemical mechanisms involved in the blocking action of members of the two series (see I, D-1, D-4), it may be postulated that the 2-methyl- and certain other 2-substituted-phenoxyethyl radicals are effective on the basis of some structural (steric) relationship to the locus of blockade. Whether anything is to be gained by a comparison of the structure of the phenoxyethylamines to that of the phenylethylamine sympathomimetics is debatable. There are many points, such as the high effectiveness of the 2-substitution, which weigh against the pharmacodynamic significance of such a comparison (see summary).

Mercapto homologs of several of the above phenoxyethylamines have also been reported to possess adrenergic blocking activity (58). The sulfur containing compounds are somewhat weaker and have a shorter duration of action than the oxy-congeners. A similar potency relationship was found for phenoxyethyl and phenylthioethyl derivatives of Dibenamine (288).

*2 Sympathomimetic amines* The first demonstration of inhibition of the effects of one sympathomimetic amine by another probably was the observation of Abderhalden and Slavu (1) in 1909 that *d*-epinephrine protected mice against the lethal effects of *l*-epinephrine. Since that time many workers have reported competitive interactions between various sympathomimetic amines (see 159, 250). The weak adrenergic blocking activity of several phenylethylenediamines and phenylhydrazines (27, 42) has been attributed to a similar action on the basis of a "structural relationship" to phenylethylamine. Recently, apparently specific inversions of the pressor response to Vonedrine ( $\beta$ -phenyl-N-propylmethylamine) by Paredrine ( $\beta$ -(*p*-hydroxyphenyl)-isopropylmethylamine) (9), and of the response to several aliphatic amines by ephedrine (8) have been reported. These reversals apparently involve a true peripheral vasodilatation not detectable in the absence of the inverting amine. Most observations indicate

of the marked uterine stimulation which they produce (see 15), and a number of compounds of this type are included in the Fourneau series. They may be considered to be chemically related to the benzodioxanes (compare structure and activity of 928F, 930F, etc., (18, 47)). These compounds appear to have many and varied pharmacological properties (see 40 for the details of older work). Several members of the series possess a hypotensive action and antagonize the pressor and renal vasoconstrictor actions of epinephrine (57, 210). Whether the hypotensive action is due entirely or even in major part to adrenergic blockade has not been determined. The adrenergic blocking action is very weak and many members of this series reduce, but do not reverse the pressor response to epinephrine. In low concentrations, many phenoxyethylamines sensitize to adrenergic stimuli. The blocking action of even the most active phenoxyethylamines is of short duration and exhibits tachyphylaxis (295).

Various members of this series have been shown to exert antidiuretic (434), central nervous system depressant (49, 210), direct vasopressor (50, 57, 210) and ganglionic effects (17, 50). They also produce dilatation or constriction of the bronchi (234), stimulate the isolated intestine (41), and inhibit epinephrine-induced relaxation of bronchi (234) and of isolated rat intestine (219). The effects of acetylcholine on bronchi (234) and of acetylcholine and barium on isolated intestine are also inhibited (41, 220, 221). Compounds which most effectively antagonize responses to barium and acetylcholine are not necessarily the most potent inhibitors of responses to epinephrine, but responses to acetylcholine and barium *in vitro* appear to be antagonized by lower concentrations than are required to block epinephrine-induced intestinal relaxation (57).

The phenoxyethylamines (929F and 1081F), both with disubstituted phenyl radicals, have been reported to block epinephrine relaxation of the dog intestine and the nonpregnant cat uterus and to reduce only slightly the pressor response to epinephrine (43, 48). Although it is true that the non-pregnant cat uterus provides a test of adrenergic inhibitory responses involving fewer complications than the intestine, 1081F directly stimulates the uterus. In the absence of experiments with non-adrenergic agents to determine the specificity of the above inhibition, these observations cannot be considered as proof of specific blockade of an adrenergic inhibitory response. Although phenoxyethyl substitutions in the  $\beta$ -haloalkylamine series usually yield highly active blocking agents (288), all compounds with double substitutions on the aromatic ring are inactive (281). Although this evidence is indirect, it suggests that disubstituted phenoxyethyl radicals do not favor the production of adrenergic blockade.

The glycemic response to epinephrine may be blocked by phenoxyethylamines (233), but this effect does not parallel the potency of members of this series in blocking vascular responses to epinephrine (33).

Phenoxyethylamines also have a negative inotropic action on the frog heart and have been reported to antagonize, but not to reverse the action of epinephrine thereon (59). The important role of direct myocardial depression in determining the cardiac effects of phenoxyethylamines is indicated by the fact that a member of this series, 1262F, suppresses arrhythmias due to electrical stimula-

that Dibenamine and Priscol do not unmask significant vasodilator responses to Vonedrine and the aliphatic amines (9, 294), although slight depressor activity was observed when relatively large doses were administered to cats pretreated with a Dibenamine congener (81)

In common with many adrenergic blocking agents, most sympathomimetic amines appear to potentiate adrenergic stimuli at low concentrations and to inhibit such stimuli at high concentrations (133, 203). Whether or not the interactions between sympathomimetic amines mentioned above involve a mechanism similar to that involved in adrenergic blockade by such agents as Priscol and Dibenamine cannot be stated at the present time.

*3 Isoquinolines* An extensive series of tetrahydroisoquinolines has been studied recently and certain members found to produce inhibition and reversal of the pressor response to epinephrine (60, 113, 195, 196). Many of the compounds also produce a considerable fall in blood pressure, apparently due to direct vasodilatation. Other members of the series, particularly the secondary amines, have a pressor action (113). N-aliphatic substituted compounds appear to have maximal adrenergic blocking activity. Such compounds may be considered to represent N-benzyl-N-alkyl- $\beta$ -chloroethylamines in which the  $\beta$ -haloalkyl group has condensed with the 2-position of the aromatic ring. However, this structural comparison appears to have no pharmacodynamic significance, because the activity of tetrahydroisoquinolines is completely abolished by N-benzyl or N- $\beta$ -chloroethyl substitutions (288). These substitutions yield compounds related in the same way to other active members of the  $\beta$ -haloalkylamine series (N,N-dibenzyl- $\beta$ -chloroethylamine and N-benzyl-di( $\beta$ -chloroethyl)amine). The blocking action of the tetrahydroisoquinolines is also entirely different from that observed in the  $\beta$ -haloalkylamine series. The former is much shorter in duration and is unaltered by thiosulfate (288, 295). Structural comparison of these reduced isoquinolines with cyclized  $\beta$ -phenylethylamines is possible (113), but probably of no greater pharmacodynamic significance than the above comparison with the  $\beta$ -haloalkylamines.

*4 Miscellaneous synthetic compounds* Morphothebaine-dimethylether (an aporphine derivative related to bulbocapnine) appears to have quite specific adrenergic blocking activity (154). The pressor response to epinephrine in anesthetized cats and the epinephrine-induced contraction of the isolated rabbit uterus and spleen are readily reversed, although the effects are very transient. The chronotropic action of epinephrine on both the frog and rabbit heart is also inhibited. However, the agent is a strong myocardial depressant and the cardiac blockade may be non-specific. It is also a relatively strong central nervous system stimulant. Morphothebaine-dimethylether does not effectively block adrenergic inhibitory responses. Epinephrine-induced relaxation of the non-pregnant cat uterus and of the rabbit ileum *in vitro* is not significantly altered by concentrations well above those shown to block excitatory responses to epinephrine.

Pyridine (326) and  $\beta$ -ionone (99) have been reported to possess adrenergic blocking activity, but their actions are quite weak. The orthostatic hypotension

induced by pentaquine (122, 334) appears to be due to depression of the central nervous system similar to that demonstrated for pamaquine (270), rather than to a "sympatholytic" effect, the latter is ruled out by failure of the drug to alter responses to epinephrine (334)

A group of unrelated drugs including atropine, diphenhydramine, meperidine, procaine and quinidine has been shown to antagonize both the vasoconstrictor and vasodilator (after Priscol) responses to epinephrine in the perfused rabbit ear (61). However, most of these agents are equally or more effective against responses to acetylcholine and histamine. Their adrenergic blocking action therefore appears only at concentrations which suppress the over-all reactivity of the effector cells.

## VII CLINICAL AND EXPERIMENTAL APPLICATIONS OF ADRENERGIC BLOCKING AGENTS

A number of clinical and experimental uses of the adrenergic blocking agents were discussed in the preceding sections for the information they provided regarding the basic pharmacology of the compounds under consideration. In this section several additional applications will be discussed briefly. Particular attention will be paid to those which involve or might be expected to involve actual adrenergic blockade. However, certain uses which appear to depend primarily upon actions other than adrenergic blockage will be included in order to place them in proper perspective.

### *A Human pharmacology*

The rational clinical use of any drug is dependent upon a clear understanding of its pharmacological actions in man. In the field of adrenergic blockage, qualitative differences between the responses of man and laboratory mammals have not been demonstrated. However, certain important quantitative differences exist. These arise particularly from the fact that unpleasant and even dangerous side-effects prevent the administration of many agents in doses adequate to produce the effects commonly seen in animal experimentation. Unfortunately, few clinical investigators have actually tested for adrenergic blockage in man. Consequently, many observations have been attributed to adrenergic blockade or "sympatholysis" even though the doses of blocking agent employed appear to be completely inadequate to produce such an effect. Only Dibenamine (183) and Priscol (151) have been conclusively demonstrated to produce significant adrenergic blockade in man.

In the majority of subjects Dibenamine inhibits or reverses the pressor effects of injected epinephrine and Neo-Synephrine, of apnea, and of the cold pressor and Flack tests. The orthostatic hypotension, nasal congestion and miosis commonly observed after Dibenamine administration must also be due to adrenergic blockade as they can be accounted for by no other known properties of the agent.

Troublesome side-effects, particularly nausea and vomiting, may occur in the clinical use of Dibenamine (183, 337, 424), but some reports indicate that they

may be largely eliminated by slow administration of the agent (158, 258) or by prior sedation (281). A reported sedative action of Dibenamine (281, 379, 424) is probably secondary to the psychic effects discussed elsewhere (see I, B-2 and VII, F-2). The direct effect of Dibenamine on the central nervous system is primarily stimulant. Orthostatic hypotension is always observed, but cannot be considered as a side-effect because it is merely one expression of the desired adrenergic blockade. It may be overcome by the use of leg and abdominal binders and preliminary observations (281) indicate that patients may compensate for postural changes in the presence of continued blockade. Presumably this adjustment is similar to that occurring after extensive sympathectomy. However, a more thorough study of the problem is warranted.

The hydrogenated ergot alkaloids have not been demonstrated to produce any extensive adrenergic blockade in man. Plethysmographic studies (37, 38, 167) have demonstrated increases in limb and digit volume and pulse, and an augmented peripheral blood flow after dihydroergocornine, but central vasomotor depression rather than adrenergic blockade appears to be the most plausible explanation for the observed changes. A central site of action is also strongly suggested by the fact that reversal of the pressor response to injected epinephrine has been demonstrated only with difficulty (large doses of dihydroergokryptine vs. very small doses of epinephrine), whereas orthostatic hypotension is readily produced (121). Inasmuch as responses to circulating epinephrine are always more readily inhibited by the ergot alkaloids than are those to sympathetic nerve activity, inhibition of vasomotor reflexes cannot be considered as proof of the establishment of adrenergic blockade if responses to epinephrine are unaltered.

Nausea, vomiting and general malaise are the most commonly observed toxic responses to the dihydro alkaloids (34, 36, 37, 121, 167) and seriously limit the dose which may be administered. These reactions appear with total doses as low as 0.3 mgm of dihydroergocornine (34, 36). This observation suggests a stronger emetic action than is observed with dihydroergotamine which is employed in considerably larger doses in the therapy of migraine.

Adrenergic blockade produced in man by Priscol (151) appears to be less complete than that produced by Dibenamine, but greater than that observed with the ergot alkaloids. The pressor response to moderate doses of epinephrine is reversed, but pressor responses to the cold-pressor and breath-holding tests are only partially inhibited and rarely reversed. Mydriasis rather than miosis may develop. The orthostatic hypotension observed after administration of Priscol is not necessarily due to adrenergic blockade, although this factor is probably involved. It is well known that orthostatic hypotension may be produced by many "non-sympatholytic" effects of drugs including central or ganglionic interruption of vasomotor reflexes and direct peripheral vasodilatation. The fact that peripheral vasodilatation is elicited in man with much smaller doses of Priscol than are necessary to reverse the pressor responses to even small doses of epinephrine (184), and that dilatation may occur in some sympathectomized limbs (151, 236) suggest that direct non-adrenergic dilatation of blood

vessels is a very important factor in the action of Priscol in man. Although the case records reported are incomplete, sympathetic regeneration could hardly have occurred in some of the cases in which dilatation was observed after Priscol. Failure to demonstrate similar vasodilatation in other sympathectomized extremities appears to be due, at least in many cases, to a high degree of pretreatment dilatation. Also, reductions in the blood pressure of extensively sympathectomized, but still hypertensive patients in response to Priscol are essentially the same as observed in non-sympathectomized hypertensives (151). The response to Priscol after sympathectomy warrants further study.

Side-effects, including some severe reactions, are relatively common with what are now considered to be effective doses of Priscol (28, 151, 236, 363, 427). The most commonly observed side-effects are piloerection, chills, nausea, vomiting, apprehension and palpitation. A severe tachycardia is noted with over-dosage (272).

### *B Peripheral vascular disease*

Among the most obvious clinical indications for adrenergic blockade are peripheral vascular conditions involving a component of sympathetically mediated spasm. Members of the  $\beta$ -haloalkylamine, ergot and imidazoline series have been employed in such conditions and all appear to produce beneficial vasodilatation. However, a precise evaluation of the relative effectiveness of these agents and their relation to other methods of therapy is impossible at this time. Most of the reported series are small, and controls and placebos have rarely been employed. From the standpoint of interpretation of results, an almost equally serious omission in most studies is the absence of evidence that significant adrenergic blockade was achieved and was responsible for the observed results.

Dibenamine has been reported to produce peripheral vasodilatation and clinical improvement in Buerger's and Raynaud's disease, acute peripheral arterial occlusion and frostbite (2, 79, 183, 379). Results with this agent appear to be due to true adrenergic blockade, but the number of cases reported to date are too few to give a clear picture of its clinical usefulness. The prolonged action of members of the  $\beta$ -haloalkylamine series may prove to be a distinct advantage in their therapeutic use.

The vasoconstrictor action of the naturally occurring ergot alkaloids is too strong for these agents to be useful in peripheral vascular insufficiency. However, their dihydro derivatives have had preliminary trial. Dihydroergotamine has been reported to produce beneficial results in peripheral vascular disease (199, 230), but its effectiveness has been questioned by some (379). Pharmacological data (see II, C-1, D-1) indicate that derivatives of members of the ergotoxine complex should have a considerable advantage over dihydroergotamine because of their lower vasospastic and greater adrenergic blocking potencies. However, it has been reported (34) that dihydroergocornine does not benefit "pathological spasm" such as that observed in Raynaud's disease, whereas dihydroergotamine has been reported to give excellent results in the treatment of this condition (230). Discrepancies such as this should emphasize

the necessity for caution in evaluating reports of the clinical effects of adrenergic blocking agents which do not include rigorous controls

Priscol has been employed by many investigators in Europe to produce vasodilatation in a variety of peripheral vascular conditions including cerebral vascular accidents (see 52, 236, 336, 363, 412, 438), with some beneficial results reported in all series. However, many of the reports are rather uncritical and placebos and controls are rarely included. It is not clear why migraine should be included in the list of conditions benefited by this vasodilator (412) (see VII, F-1). Only one full report of the clinical use of Priscol (151) has appeared in the American literature. In this study the agent was found to produce significant peripheral vasodilatation, much of which appears to be independent of adrenergic blockade. The results indicate that larger doses than employed in many of the earlier studies are necessary to achieve significant effects. Patients with Raynaud's, Buerger's and arteriosclerotic peripheral vascular disease, with acute vascular occlusion and with "causalgia" were reported to be benefited by Priscol. The most favorable results were observed in Raynaud's disease and the least favorable results in causalgia.

### C *Pheochromocytoma*

The diagnosis and preoperative therapy of pheochromocytoma are obvious, although rare, indications for the use of adrenergic blocking agents. The benzodioxanes have been employed successfully in the diagnosis of several cases, and in the detection of unsuspected multiple tumors either during or after operation (64, 134, 143). However, blockade produced by the benzodioxanes is much too short to permit these agents to be used therapeutically, also, unpleasant side-effects are not uncommon. Dibenamine has been employed effectively in the diagnosis and in the rather prolonged preoperative maintenance of patients with pheochromocytoma (376, 379). The results reported have been excellent with injections at 72 hour intervals providing complete symptomatic relief. During the period of Dibenamine administration the Roth-Kvale histamine test was found consistently to be negative, although it was highly positive prior to treatment with Dibenamine.

### D *Hypertension*

Many workers studying adrenergic blocking agents have entertained the thought that these compounds might be of value in the treatment of "essential" hypertension. In the absence of any reliable information regarding the etiology of essential hypertension this approach is without theoretical basis. However, reports of partial, although highly variable, relief obtained from surgical sympathectomy in this condition have kept alive the hope that chemical adrenergic blockade might provide similar benefits.

1 *Experimental neurogenic hypertension* The role of adrenergic blockade in the therapy of neurogenic hypertension is obvious. Consistent, though transient, lowering of blood pressure has been observed in hypertensive dogs administered ergotamine (191), 883F and 933F (29, 192). However, central inhibition of



vasomotor activity as well as adrenergic blockade may be involved in the observed results. Compounds 1071F and 1072F, adrenergic blocking agents with little effect on the central nervous system, are not effective in decreasing pressure in chronic neurogenic hypertension (29).

*3 Experimental renal hypertension.* The role of adrenergic factors in experimental renal hypertension is vague. The sequence of events by which interference with renal hemodynamics leads to elevation of the systemic blood pressure has been carefully studied and has been shown to be independent of nervous mechanisms (53, 142). However, the many similarities between experimental renal hypertension and human essential hypertension (see 306, 234a) have prompted extensive investigation of the former. Renal hypertensive animals respond to sympathectomy by a limited reduction in blood pressure (13), but prior sympathectomy does not prevent the development of renal hypertension (13, 119, 193, 406). There is no reason to believe that, in either experimental renal or human essential hypertension, adrenergic blockade can accomplish more than surgical excision of the sympathetic nervous system.

Adrenergic blocking agents produce a significant, but highly variable reduction in systemic arterial pressure in animals with experimental renal hypertension. Yohimbine administered orally for periods up to 35 days elicits some reduction of the blood pressure in dogs with chronic renal hypertension (200). However, the pressure is not consistently returned to normotensive levels. Dibenamine administered intravenously at three-day intervals to dogs with chronic renal hypertension has been observed to produce some reduction in pressure, but not to lower it consistently into the normal range (423). Wide fluctuations in pressure were noted during the period of treatment. In these experiments with Dibenamine the degree of adrenergic blockade was tested at intervals and the failure of Dibenamine to lower the pressure consistently to normotensive levels does not appear to be due to inadequate adrenergic blockade. Daily oral administration of Dibenamine to renal hypertensive rats in doses adequate to produce partial, but not complete, adrenergic blockade produced similar results (284). All hypertensive animals (and also normotensive controls) responded to each administration with a rapid but limited reduction in blood pressure. However, only 65% of the hypertensive and none of the normotensive animals showed a cumulative effect. Pressures of the animals which responded favorably were lowered to essentially normotensive levels which were irregularly maintained during 10 days of drug administration and for three to five days thereafter. The observed effects were shown to be due to adrenergic blockade because they could not be duplicated with 2-dibenzylaminoethanol (the hydrolysis product of Dibenamine) which has many pharmacological properties in common with Dibenamine but lacks adrenergic blocking activity.

Single injections of 883F and 933F (29, 100, 212) produce only irregular vasodepression in dogs with chronic renal hypertension, a response very similar to that seen in normotensive controls. Single injections of pentobarbital or yohimbine (330), and 883F, but not 933F (357), have been reported to produce a transient depressor response in renal hypertensive rats, which appeared to be

greater in animals hypertensive for more than two months. These observations on rats, particularly the difference in response to 883F and 933F, have been interpreted to indicate that neurogenic factors are of importance in late, but not in early renal hypertension. Fewer rats with prolonged renal hypertension (over two months duration) responded to Dibenamine with a persistent lowering of the blood pressure than did those with a shorter period of hypertension (284). Observations demonstrating an almost equal effect of 933F and 883F in neurogenic hypertension (see VII, D-1) have apparently been overlooked in this interpretation. It has been noted that single injections of ethyl-yohimbine cause a marked reduction in the blood pressure of renal hypertensive dogs under pentobarbital anesthesia (68). The reductions in pressure induced by pentobarbital and yohimbine therefore appear to be due to cumulative factors and attributing them to a common inhibition of sympathetic vasoconstrictor activity does not appear to be warranted at the present time.

The above observations, particularly those obtained with single injections of blocking agent, are extremely difficult to evaluate. However, their marked irregularity, compared with the consistent depressor response to adrenergic blockade seen in animals with neurogenic hypertension, argues against derangement of sympatho-adrenal function as a major factor in renal hypertension. It must be concluded that the contention that nervous factors are of importance in late but not in early renal hypertension (302, 330, 357) has not received convincing support from experiments employing adrenergic blocking agents.

It is known that the blood pressure can be maintained within normal limits after complete sympathectomy (152, 257), presumably by autonomous vascular tone. The possibility must therefore be considered that the rate and extent of peripheral vascular compensation rather than the level of sympathetic activity may determine the magnitude and duration of the depressor response to drug-induced sympathetic blockade.

3 *"Essential" hypertension.* The clinical application of adrenergic blockade to the study and treatment of hypertension has been very limited. Therapy with Dibenamine has been reported to produce significant benefit in severe, particularly malignant, hypertension (424). The drug was found to lower significantly the blood pressure in most cases, and relief of symptoms such as hypertensive encephalopathy was even more prominent. Other workers (158) have observed a significant depressor response to Dibenamine, lasting 24 to 72 hours, in early benign hypertension, but not in patients with advanced organic changes in the cardiovascular system. On this basis it has been suggested that the response to this drug be determined prior to sympathectomy as a measure of the role of the sympatho-adrenal system in a given case of hypertension. On theoretical grounds, an agent with the specificity of Dibenamine would be expected to be ideal for this purpose, indeed, attention has been called to the similarity between the effects of Dibenamine medication and those of surgical sympathectomy (423). However, the same arguments regarding possible misinterpretation of depressor responses to sympatho-adrenal blockade apply here as in the above discussion of responses to adrenergic blockade in experimental renal hypertension. Results obtained with Prisol, the eight alkaloids, tetra-

ethylammonium and spinal anesthesia where factors in addition to blockade of the sympatho-adrenal system are involved would seem to have even less diagnostic specificity. It has been reported that Dibenamine is superior to tetraethylammonium as a test for predicting the results of sympathectomy in acute peripheral vascular conditions (79), but the same worker questions its prognostic value in hypertension. Lack of knowledge concerning the etiology of essential hypertension makes the interpretation of any prognostic test extremely hazardous.

Dihydroergotamine (377) and dihydro derivatives of members of the ergotoxine complex (36, 37, 121, 167) have recently been studied in a few cases of hypertension. Parenteral administration appears to produce a significant reduction in blood pressure and an orthostatic hypotension, but the response of different patients varies widely and as yet unpredictably. Reduction in the elevated blood pressure was not found to parallel suppression of experimentally initiated vasomotor reflexes, and in many cases (36, 38) an increase in the dose of dihydroergocornine caused an increase rather than a decrease in pressure. A similar pressor response to large doses of dihydroergocornine has been noted in normotensive individuals (36). This alteration in response with increasing dosage is compatible with a central site of action. Systolic pressure is often significantly reduced without any change in diastolic pressure (see 37), a fact which questions relaxation of peripheral arteriolar constriction as a significant factor in the depressor response. More than 10 times the parenteral dose must be employed when the dihydro ergot alkaloids are administered orally and they appear to produce even more irregular responses when administered by this route (36, 121).

The hypotension and depression of cardiovascular reflexes observed in man are undoubtedly due to central nervous system rather than "sympatholytic" effects. Central depression of vasomotor reflex pathways and central vagal stimulation may both be involved. Bradycardia is usually observed.

Nausea and vomiting are common after the administration of dihydro ergot alkaloids to hypertensive patients (36, 121, 167) and are not necessarily associated with blood pressure changes. Total doses as low as 0.3 mgm dihydroergocornine have been reported to produce rather severe side-effects (34, 36). It has been stated that nausea and vomiting result from lower doses of dihydroergocornine in hypertensive than in normotensive individuals (36).

Priscol has been critically tested in only a small number of hypertensive patients (151), even large doses were found to produce very little reduction in blood pressure. The extent to which adrenergic blockade is involved in the cases showing some reduction is called into question by the fact that essentially equal responses occurred in patients who had remained hypertensive after extensive sympathectomy. Even a massive overdose of Priscol fails to reduce the blood pressure (272), presumably because cardiac stimulation balances peripheral vasodilatation. Some blood pressure reduction has been reported to result from the administration of Priscol to a few hypertensive patients (363) but this observation has not been substantiated.

The few observations which have been made indicate that the benzodioxanes

933F and 1164F tend to raise the blood pressure in human essential hypertension (143), probably due to central vasomotor stimulation (see IV, B-3)

### *E Cardiac effects*

Although Dibenamine, Priscol, yohimbine, corynanthine, the ergot alkaloids and several benzodioxanes have all been shown to inhibit adrenergically induced cardiac arrhythmias in hearts sensitized by anesthetics and other hydrocarbons (12, 96, 135, 269, 293, 298, 299, 305, 370, 372), only Dibenamine has been studied clinically (283). This agent proved to be very effective in preventing "spontaneous" arrhythmias in surgical patients under deep cyclopropane anesthesia, a fact which indicates an etiological role of adrenergic stimuli in these arrhythmias.

The effects of various blocking agents on cardiac arrhythmias induced by procedures or conditions not involving adrenergic stimuli is quite variable. Both 883F and 933F have been found to be completely ineffective in preventing cardiac arrhythmias caused by digitalis overdosage in cats (104), but to prevent those due to electrical stimulation and  $\text{BaCl}_2$  (96). A phenoxyethylamine derivative (1262F) protects against fibrillation caused by several non-adrenergic stimuli (51, 97), apparently on the basis of direct myocardial depression. Ergotamine and dihydroergotamine provide significant protection against fatal ventricular fibrillation after acute coronary occlusion in dogs (251, 295). However, the protection provided by the dihydro ergot alkaloid does not increase in proportion to its adrenergic blocking potency and Dibenamine is ineffective (295). These observations strongly suggest that the protection observed is dependent upon non-specific myocardial effects of the ergot alkaloids rather than upon adrenergic blockade. In addition, dihydroergotamine is less than one-half as potent as ergotamine in preventing epinephrine-cyclopropane arrhythmias (305), a fact which suggests that the limited protection afforded by the ergot alkaloids against this type of arrhythmia (12, 298, 305) is also largely independent of adrenergic blockade (see discussion, 298).

Other cardiac effects of ergotamine and dihydroergotamine observed with doses obviously too small to produce adrenergic blockade must be dependent upon direct myotropic or central nervous system actions. In small doses these alkaloids cause an increase in the T waves of the human electrocardiogram (165, 301, 378, 416) and also inhibit changes which appear when the subject is standing (362, 418). They have been used diagnostically to enhance disturbances in cardiac rhythm in acute rheumatic fever (417), and to differentiate between organic and functional heart disease, particularly by preventing ECG changes in the latter in response to exercise or anoxia (30, 256, 301, 378). It has been argued that ergotamine or dihydroergotamine affects the ECG by a "sympatholytic" action which alters "autonomic balance." However, in the absence of tests to indicate the presence of adrenergic blockade it must be assumed that such small doses act through some mechanism other than "sympatholysis." This interpretation is strengthened by excellent evidence of the inability of the ergot alkaloids, even when administered in adequate blocking doses, to alter

most responses of the mammalian myocardium to adrenergic stimuli or to block adrenergic dilatation of the coronary arteries (see II, C-2, C-3) If any action of the ergot alkaloids on the autonomic nervous system is involved in the observed ECG alterations it is probably central inhibition of vasomotor reflexes or central vagal stimulation The latter factor would seem to be particularly important as alterations in the ECG are frequently accompanied by some bradycardia To claim that the effect of 0.5 mgm of ergotamine tartrate in man is "primarily sympatholytic in character" is to ignore the accumulated data on the human and animal pharmacology of this agent

### *F Miscellaneous clinical uses*

*1 Migraine* The statement that adrenergic blockade is involved in the beneficial effects of the ergot alkaloids in migraine recurs even in recent clinical literature, this misunderstanding is probably involved in attempts to employ Priscol in the treatment of migraine (412) Many diverse observations provide strong evidence against this interpretation Careful studies by Wolff and associates on the vascular mechanisms in migraine (146, 400) have demonstrated that vasodilatation rather than vasoconstriction predominates during an attack and that ergotamine acts by promoting vasoconstriction rather than vasodilatation, the latter would result if sympathetic blockade were the predominant effect This mechanism of action of ergotamine is supported by the fact that epinephrine (146) and other sympathomimetic amines (248) may also provide relief Furthermore, the doses of the ergot alkaloids employed in migraine, are much too small to produce adrenergic blockade Ergonovine is effective in a considerable percentage of cases despite its lack of adrenergic blocking activity (231) Finally, hydrogenation of the ergot alkaloids, which decreases direct vasoconstrictor and increases adrenergic blocking activity, reduces their effectiveness in migraine For example dihydroergotamine must be employed in doses about double those of ergotamine (see 124, 125, 398) and dihydroergocornine, one of the most potent adrenergic blocking agents in the series, has only questionable value in migraine (124) One report (377) claiming that dihydroergotamine has a greater potency than ergotamine in the treatment of migraine is not in agreement with most observations

*2 Psychoses* Reports of benefit from the use of ergotamine (181, 214, 310, contrast 153) and Dibenamine (258, 337) in psychiatric conditions are very difficult to interpret In most cases the criteria of improvement are nebulous However, Medinets and coworkers (258) have made a laudable attempt to quantitate the responses of schizophrenic patients to Dibenamine and have reported significant improvement

It is probable that any benefit derived from these agents in psychoses is due to factors other than adrenergic blockade, but unfortunately these have been largely overlooked in a tendency to attribute the results to autonomic "balancing" or "unbalancing" by adrenergic blockade All adrenergic blocking agents reported to be of value in these conditions have at least transient direct effects on the central nervous system The role of direct central effects is particularly

clear in the case of the ergot alkaloids. These agents always produce brain-stem depression with smaller doses than are necessary for adrenergic blockade, somnolence and sedation are among the most prominent signs of acute toxicity in primates (420), and an adrenergically inactive alkaloid (ergonovine) has been found to produce essentially the same psychic response as ergotamine (214). The extremely potent effects of the ergonovine congener lysergic acid diethylamide on the central nervous system (see II, A) may provide a useful point of departure for a critical study of the non-adrenergic psychic effects of the entire series of ergot alkaloids.

A second consideration in evaluating the role of adrenergic blockade in the psychic responses to blocking agents is that, insofar as they have been studied, adrenergic blocking agents do not inhibit responses of the central nervous system to adrenergic stimuli. This factor has been considered in only one of the reports on the subject (258). Finally, with the exception of certain studies on Dibenamine (258, 337) there is little evidence that the doses employed produce significant adrenergic blockade. In one series (310), huge doses of dihydroergotamine (to 200 mgm /day) were administered orally, but little is known of the blocking action of the ergot alkaloids when administered by this route, and no attempt was made to show that adrenergic blockade was achieved. Studies with the hydrolysis product of Dibenamine which retains the central stimulant, but is devoid of adrenergic blocking activity (see I, B-2) would be of interest in determining the mechanism involved in the production of the reported beneficial psychic effects of Dibenamine.

*3 Other uses* Although it does not alter the pressure in normal eyes, Dibenamine has been reported to reduce very effectively the intraocular pressure in cases of acute glaucoma refractory to other therapy (75). It is known that cervical sympathectomy causes some lowering of intraocular pressure in animals (202), but the mechanism of the Dibenamine action is far from clear. The reduction in pressure is much greater than can be accounted for on the basis of the miosis produced.

Dibenamine has been observed to provide marked symptomatic relief in cardiospasm, accompanied by roentgenographic evidence of relaxation of the cardia and rapid emptying of the esophagus (281). However, more extensive observations are necessary to determine the duration of relief and the incidence of favorable responses. It has been reported that dihydroergotamine also may relieve cardiospasm (199), but these observations are complicated by the simultaneous administration of physostigmine.

Dibenamine also induces a significant diuresis and an increased urea clearance in patients with malignant hypertension (424). Priscol has been reported to produce a similar response in acute nephritis (227).

#### *G Miscellaneous experimental applications*

*1 Shock* Early in the study of the  $\beta$ -haloalkylamine blocking agents it was noted that less hemorrhage is required to induce a fall in blood pressure in Dibenamine-treated animals than in controls, but that equal or larger withdrawals

of blood could be made from the former before shock supervened (297) More detailed experiments (331, 422) have now demonstrated that adrenergic blockade with Dibenamine provides marked protection against both hemorrhagic and traumatic shock Careful hemodynamic measurements in control and Dibenamine-treated dogs subjected to hemorrhagic and traumatic shock indicate that the observed protection is largely due to the elimination of reflex vasoconstriction which ordinarily sustains blood pressure at the expense of blood flow (331) The protection afforded by Dibenamine under these conditions might have been anticipated on the basis of older experiments (120) demonstrating a marked resistance of sympathectomized dogs to hemorrhagic shock

2 *Inhibitory properties of sympathomimetic amines* Adrenergic blocking agents have been employed as tools in several studies designed to evaluate the relation between the chemical structure of sympathomimetic amines and the depressor component of their activity The effect of a Dibenamine congener (N-benzyl-N- $\beta$ -phenylisopropyl- $\beta$ -chloroethylamine) on the pressor response to 20 pressor amines and catechol has recently been reported (81) All agents except Neo-Synephrine, Privine and Paredrinol elicited some depressor response after blockade Maximum adrenergic blockade may not have occurred in these experiments Two-thirds of the preparations showed only partial inhibition of response of the nictitating membrane to epinephrine Other workers, most of whom have studied only a few amines, have observed much less depressor response after adrenergic blockade, particularly with non-catechol, aliphatic and imidazole sympathomimetic agents (11, 105, 114, 259, 260, 408, 409) However, one report indicates epinephrine-like reversal even of Privine and Neo-Synephrine by a  $\beta$ -haloalkylamine (414) The relatively large doses of pressor amines employed by Coret (81) may have been a factor in the production of the observed depressor responses, for it has been noted that large doses of several amines are reversed more readily than small doses (114, 294, 414) and that repetition of small doses produces a similar increased depressor effect (294) A standardization of methods and doses will be necessary before the above divergent results can be quantitatively interpreted

Another study of cardiovascular responses to an extensive series of sympathomimetic amines administered after large doses of Dibenamine (287, 294) has indicated that four structural factors are of importance in eliciting a depressor response alkyl substitution on the amine, hydroxyl substitutions (especially 3, 4-) on the aromatic ring,  $\beta$ -aliphatic substitutions and  $\alpha$ -oxy substitutions The importance of these factors tends to decrease in the order listed, but at least two of the mentioned substitutions are necessary for any considerable depressor activity, and the alkyl groupings are relatively ineffective in the absence of the phenolic hydroxyls Qualitative confirmation of the significance of these constituents is found in several of the series of experiments discussed in the preceding paragraph when they are reevaluated with these factors in mind

A study of cardiovascular responses to graded doses of epinephrine in cats before and after the administration of Dibenamine has provided data for the construction of dose-effect curves for pressor and depressor responses (286)

Differences in the shape of these curves may provide an explanation for recognized differences in the response of untreated animals to large and small doses of epinephrine and perhaps other sympathomimetic amines

*3 Identification of adrenergic mediators* A number of workers have employed adrenergic blocking agents as tools to unmask the depressor component of the response to epinephrine and thus to differentiate this agent from norepinephrine which has little or no vasodepressor action. Studies have been carried out particularly in efforts to identify the sympathetic mediator and the sympathomimetic agents extracted from various tissues. The ergot alkaloids have been most commonly employed (108, 132, 419), probably because of their availability, benzodioxanes have also been used (264). However, Euler has recently employed Dibenamine for this purpose (109) with more reproducible results and fewer complicating side-effects. The depressor responses noted after ergotamine, and more recently after Dibenamine, also have been studied as one step in the characterization of newly synthesized series of sympathomimetic agents (*e.g.*, 392).

After confirming the specificity of the adrenergic blocking action of Dibenamine, Folkow and coworkers have employed this agent in several extensive studies of the mechanisms of certain cardiovascular reflexes and of the relative roles of cholinergic and adrenergic factors in sympathetic vasodilatation (114, 115, 116, 117).

*Other uses* The  $\beta$ -haloalkylamines have also been utilized by a number of investigators to determine whether the effects of various agents and procedures are adrenergically mediated. This question has been answered in the affirmative for the pressor effect of certain anticholinesterases (80), the secondary pressor response to epinephrine (229), sweating in certain areas (palms of hands, etc.) (156, 183, 258) and the hyperthermic response to typhoid toxin (415). The inhibition of hyperthermia by N-1-naphthylmethyl-N-ethyl- $\beta$ -bromoethylamine was similar to, but somewhat less than, the inhibition by corynanthine and 883F of dinitrophenol hyperthermia reported many years ago (374). Dibenamine (157) and ergotamine (246) have also been employed in experiments demonstrating a direct peripheral vasoconstrictor action of nicotine.

Prisol has been used as a vasodilator to facilitate blood pressure readings in the rat's tail (74), but the validity of such determinations may be questioned.

Rapid intravenous administration of large doses of Dibenamine has been shown to prevent post-coital ovulation in the rabbit (358). It was concluded that this inhibition indicated adrenergic mediation to the anterior pituitary. However, the marked central nervous system excitation which occurred and the short time intervals involved in these experiments strongly suggest that direct central nervous system stimulation rather than adrenergic blockade was important in the observed inhibition (280). Dibenamine failed to prevent the release of adrenocorticotrophin in response to stress in experiments in which adequate time was allowed for side-effects of the agent to be dissipated (394).



## SUMMARY

Members of the various series of adrenergic blocking agents differ widely in the blockade they produce and even more widely in the nature of their side-effects. However, a general pattern of activity emerges if the components of specific adrenergic blockade are extricated from complicating side-effects, often a difficult process because of the limited nature of many reports.

The data listed in table II summarize the pharmacological properties of the more important series of adrenergic blocking agents. This tabulation is certainly not definitive and some exceptions to almost every statement in the table have been reported. Aberrant results in the field of adrenergic blockade have been frequently attributed to species differences or to qualitative differences in the activities of a few members of a series. Such differences may exist (e.g., absence of significant adrenergic vasodilatation in the rabbit), but in explaining conflicting results they should be invoked only as a last resort. Whenever well-controlled experiments with a group of related agents have been carried out in parallel on several species, many apparent exceptions have disappeared. Side-effects, use of an inadequate dose range or factors of experimental technic frequently may be the basis for apparent exceptions, and must be rigorously ruled out before observations which conflict with a general pattern of activity can be accepted.

The contraction of smooth muscle and the secretion of exocrine glands in response to adrenergic stimuli are inhibited by all adrenergic blocking agents and blockade of these responses has been tacitly accepted as the basis for defining adrenergic blocking activity. The most thoroughly studied manifestations of this action are inhibition of the pressor response to injected epinephrine and inhibition of the epinephrine-induced contraction of various smooth muscle structures *in vitro*. However, the elimination of such responses does not constitute proof of specific adrenergic blockade, many substances in adequate dosage are capable of destroying the ability of smooth muscle to contract. Reversal of the pressor response, as distinct from its depression, is more adequate evidence of specific blockade because such reversal indicates that the vascular system is still capable of physiological responses. In experiments on smooth muscle *in vitro*, reactions to several activating agents must be studied in parallel in order to establish the specificity of alterations in the response to epinephrine.

All specific adrenergic blocking agents inhibit responses to circulating mediator more readily than those to adrenergic nerve stimulation. Some agents show a much greater differential than others in their activity against these two types of stimuli, but all gradations occur and the difference is therefore unreliable as a basis for classifying blocking agents. Inhibition of vasomotor reflexes cannot be considered as proof of "sympatholytic" activity. The ergot alkaloids inhibit vasomotor reflexes more readily than responses to injected epinephrine, but the mechanism involved is depression of the brain stem rather than inhibition of responses to sympathetic nerve activity.

Specific blockade of adrenergic "inhibitory" responses does not appear to have been conclusively established for any blocking agent. Epinephrine-induced

Summary of properties of major series of adrenergic blocking agents

AGENTS		β-HALO- ALKYLAMINES	ERGOT ALKALOIDS <sup>1</sup>	INDAZOLINES	BENZODIOXANES	YOHIMBINE CORYNANTHINE	PHENOXY- ETHYLAMINES
PROPERTIES							
Adrenergic blocking actions							
Specificity <sup>2</sup>		Very high	Natural—very low DHA—low	Low	Low	High	Very low
Effectiveness <sup>2</sup>		Very high	High (DHA—more po- tent)	Low	Low	Low	Low
Duration of action		Very long	Intermediate	Intermediate	Short	Short	Short
Type of blockade		Non-equilibrium	Equilibrium	Equilibrium	Equilibrium	Equilibrium	Equilibrium
"Excitatory" responses	Organs	All	All	Ocular smooth muscle resistant	Ins resistant	All	All?
	Stimuli	Sympathomimetics > nerve impulses	Sympathomimetics > nerve impulses	Sympathomimetics > nerve impulses	Sympathomimetics > nerve impulses	Sympathomimetics > nerve impulses	Sympathomimetics > nerve impulses
Cardiac re- sponses	Mamma- lian	Unaltered but ar- rhythmias pre- vented	Unaltered	Unaltered, but arrhyth- mias prevented	Unaltered	Unaltered, but ar- rhythmias pre- vented	Unaltered
	Amphib- ian	Blocked or reversed	Blocked or reversed	Little effect	Blocked	Blocked?	Blocked?
Inhibitory <sup>3</sup> responses	Observa- tions	Little effect	Intestine and some uteri blocked or reversed, vasodilatation unal- tered	Intestine moderately in- hibited, uteri and vaso- dilatation unaltered	Intestine blocked, cat uterus and vasodilata- tion unaltered	Intestine slightly in- hibited	Intestine and some uteri blocked and reversed
	Status		Specificity not demon- strated cholinergic stimuli potentiated	Specificity not demon- strated	Specificity not demon- strated	Specificity not demon- strated	Specificity not demon- strated
Glycemic response		Unaltered or moder- ately inhibited	Strongly inhibited	Unaltered	Moderately inhibited	Moderately inhibited	Moderately inhibited
C.N.S. Responses (to symp amines)		Unaltered	?	?	Unaltered	?	?

## PHARMACOLOGY OF ADRENERGIC BLOCKADE

Actions other than adrenergic blockade				
muscle]	Little effect	Actions other than adrenergic blockade		
		Natural—many organs strongly stimulated DHA—slightly stimulated or relaxed	Parasympathomimetic and histamine like stimulation, symplethomimetic relaxation, peripheral vasodilatation	Modestly stimulated (direct and secondary to sympathetic-adrenal discharge), some or none relaxed
Central nervous system	Myocardium slightly depressed and transiently depressed	Myocardium strongly depressed, ECG altered, inhibited through the vagus, arrhythmias prevented	Strongly stimulated (sympathomimetic) coronaries dilated	Myocardium strongly depressed, inhibited through vagus, stimulated through sympathetic, arrhythmias prevented
	Strongly but transiently stimulated	Strongly and complexly stimulated and depressed, vasomotor reflexes depressed, vasomotor center depressed, vomiting induced, respiration depressed	Not significantly affected	Strongly stimulated and depressed, antidiuretic produced
		Cholinergic stimuli potentiated, pancreatic secretion inhibited	Gastro, pancreatic, salivary secretion stimulated, glycomia produced	Some cholinergic responses blocked, powerful local anesthetic
Miscellaneous	Histamine blocked by some compounds, local tissue irritation		Glycomia through sympathetic, local anesthetic	Local anesthetic

Summary of adrenergic blocking action produced

Summary of adrenergic blocking action produced

Summary of adrenergic blocking action produced

Summary of adrenergic blocking action produced

Summary of adrenergic blocking action produced

Summary of adrenergic blocking action produced

Summary of adrenergic blocking action produced

Summary of adrenergic blocking action produced

Summary of adrenergic blocking action produced

Summary of adrenergic blocking action produced

1 Properties of natural and dihydrogenated (DHA) alkaloids similar on the basis of the complications and limitations of adrenergic blocking action produced  
2 Factors such as central nervous system depression roughly weighted on the basis of the complications and limitations of adrenergic blocking action produced  
3 Ability to block responses to strong adrenergic stimuli

relaxation of certain organs is inhibited by a number of compounds. However, the interpretation of this effect is complicated by the presence of intramural parasympathetic ganglia in many organs which respond to adrenergic stimuli by relaxation, the known parasympathomimetic and direct musculotropic actions of many adrenergic blocking agents, and the ganglionic effects of epinephrine. In sharp contrast to the uniformity of blockade of excitatory responses, antagonism of inhibitory responses varies widely with different species, organs, blocking agents and experimental conditions. Two general observations regarding the inhibition of adrenergic relaxation of smooth muscle weigh against the conclusion that this is an expression of specific adrenergic blockade. (A) The responses of organs most subject to complicating reactions, (e.g., intestine) are most frequently reported to be inhibited. In contrast, relaxation of the non-pregnant cat uterus by epinephrine is relatively uniform and dependable, responses of this organ are much less frequently reported to be inhibited and only partial blockade is usually observed. Finally, blockade of adrenergic inhibition of the vascular musculature has never been clearly demonstrated. Vascular smooth muscle represents the most reliable object upon which to test blockade of adrenergic inhibitory responses because of the absence of ganglia, the absence of constrictor responses to cholinergic stimuli, etc. (B) Blockade of adrenergic inhibitory responses is reported most frequently as a property of those drugs (ergot alkaloids, phenoxyethylamines, benzodioxanes) which possess many important side-effects and least frequently as a property of those agents ( $\beta$ -haloalkylamines, yohimbine) which exhibit the greatest specificity of action. It must be concluded that blockade of adrenergic inhibitory responses by members of any of the known series of adrenergic blocking agents has not been conclusively established.

It is generally agreed that no adrenergic blocking agent specifically inhibits the chronotropic and inotropic responses of the mammalian heart to adrenergic stimuli. Even those agents which profoundly depress the myocardium have rarely been reported to depress in equal measure its response to epinephrine. In contrast to the responses of the mammalian heart, stimulation of the amphibian heart by epinephrine appears to be subject to inhibition by several agents. This inhibition may be markedly altered by the composition of the perfusion fluid, a fact which may account for a number of contradictory reports which have appeared.

In contrast to their ineffectiveness against the chronotropic and inotropic responses of the mammalian heart to epinephrine, adrenergic blocking agents are quite effective in preventing epinephrine-induced arrhythmias both in the presence and in the absence of sensitizing hydrocarbons. Some agents ( $\beta$ -haloalkylamines, yohimbine, imidazolines) apparently protect by virtue of their adrenergic blocking activity, whereas others (ergot alkaloids, benzodioxanes, phenoxyethylamines) protect by directly depressing the myocardium. Compounds of the latter type are also effective against arrhythmias evoked by non-adrenergic stimuli (electric current,  $\text{BaCl}_2$ , etc.) whereas the former are not.

Many adrenergic blocking agents inhibit the glycemic response to epinephrine, but other potent agents, notably Dibenamine and Priscol, have little effect on this response. Among agents for which adequate data are available it is obvious

that inhibition of the glycemic response is very poorly correlated with the inhibition of excitatory responses of smooth muscle. In addition substances other than adrenergic blocking agents (e g, posterior pituitary) are able to inhibit the hyperglycemic response to epinephrine. The fact that the antiglycemic action of ergotovine is additive with that of posterior pituitary suggests that specific adrenergic blockade is not the basis for the observed inhibition.

The blockade produced by all major series of adrenergic blocking agents is relatively specific for adrenergic stimuli. Lack of specificity is manifested primarily by direct actions on various tissues. Such direct actions may seriously interfere with the use of these agents both in experimentation and in therapy, by producing effects which cannot be readily differentiated from those of adrenergic blockade or by preventing the administration of doses adequate to establish adrenergic blockade. Direct actions of the natural ergot alkaloids, the benzodioxanes and the phenoxyethylamines on smooth muscle not infrequently complicate experiments or produce toxic reactions. However, the most serious side-effects are those involving the central nervous system. For example, all ergot alkaloids depress vasomotor centers and inhibit transmission of vasomotor reflexes through the brain stem in smaller doses than are required to produce sympathetic blockade. In addition, stimulation of the emetic center by the ergot alkaloids is responsible for the fact that "sympatholytic" doses of these agents cannot be tolerated by man. Central nervous system stimulation is also the most prominent side-effect of the  $\beta$ -haloalkylamines. However, this has much less serious consequences than in the case of the ergot alkaloids because the adrenergic blockade produced by the  $\beta$ -haloalkylamines far outlasts the central effects and the latter do not significantly interfere with vasomotor tone or reflexes.

Little is known regarding the finer mechanisms of action of any of the adrenergic blocking agents. Evidence is available to indicate that the  $\beta$ -haloalkylamines act through an alkylating reaction with cellular constituents, but nothing is known concerning the nature of these constituents or why their alkylation should prevent the contraction of smooth muscle or the secretion of exocrine glands in response to specific stimuli.

Present data indicate that alterations in the destruction or transformation of the mediator *in vivo*, and alterations in cell permeability are not important factors in adrenergic blockade. A dynamic equilibrium between mediator and blocking agent is characteristic of at least certain stages in the action of all types of compounds producing adrenergic blockade. However, the question why certain chemical structures and not others produce effective blockade remains unanswered.

The steric antagonism of a pharmacologically active compound by a relatively inactive congener is a common observation and such a mechanism may be involved in the antagonism of one sympathomimetic amine by another. However, almost insurmountable obstacles are encountered in efforts to explain the action of the more effective blocking agents on the basis of a molecular similarity to the basic phenylethylamine structure of sympathomimetic agents.

All the most active and specific blocking agents are found in series ( $\beta$ -halo-

alkylamines, yohimbine, ergot) where the relationship to phenylethylamine is the least obvious. In the  $\beta$ -haloalkylamine series, benzyl and phenoxyethyl derivatives are active, but the phenylethylamine derivatives are almost completely inactive. The relationship of the benzodioxanes to phenylethylamine is much less obvious than that of the phenoxyethylamines, and yet the former blocking agents are much more effective and specific than the latter. Some workers have considered the presence of a phenylalanine residue in ergotamine to allow a structural comparison with phenylethylamine. However, replacement of this radical by a non-aromatic amino acid residue may increase rather than decrease blocking activity, e.g., ergokryptine. Additional objections to the acceptance of structural similarity to phenylethylamine as a basis for most adrenergic blocking activity may be found in the preceding sections. At the present time little is gained by attempts to squeeze blocking molecules into the phenylethylamine mold.

Discovery of the adrenergic blocking activity of the  $\beta$ -haloalkylamines and imidazolines, and preparation of dihydroderivatives of the ergot alkaloids have done much to renew interest in the field of adrenergic blockade. No ideal blocking agent is yet available, but compounds with improved specificity and potency are rapidly being developed in all three series. Future experimental and clinical applications of these agents may be expected to yield new and valuable information on the normal and pathological physiology of the sympatho-adrenal system.

#### REFERENCES

1. ABERHOLDEN, E., AND SLAVU. Weitere Studien über das physiologische Verhalten von l, d und dl-Suprenin. III Mitteilung. Ztschr f Physiol Chem, 59: 129-137, 1909.
2. ABRAMSON, D. J. Clinical evaluation of several medical procedures used in the treatment of common peripheral vascular disorders. Am Col Physicians Meeting Milwaukee, Wis., 1947.
3. ABREU, B. E., LIDDLE, G. W., HANDLEY, C. A., AND ELLIOTT, H. W. Preliminary observations on the influence of ergotamine and dihydroergotamine on cerebral blood flow in the dog. Federation Proc, 6: 304, 1947.
4. ACHESON, G. H., AND FARAH, A. Personal communication.
5. ACHESON, G. H., FARAH, A., AND FRENCH, G. N. Some effects of dibenamine on the mammalian heart. Federation Proc, 6: 305, 1947.
6. ADLER, H. F., ATKINSON, A. J., AND IVY, A. C. Supplementary and synergistic action of stimulating drugs on the motility of the human colon. Surg, Gynec & Obst, 74: 809-820, 1942.
7. AGAR, W. T. The action of adrenaline upon the uterus of the guinea pig and its modification by eserine. J Physiol, 98: 492-502, 1940.
8. AHLQUIST, R. P. The sympathomimetic vasodilating action of the aliphatic amines. J Pharmacol & Exper Therap, 85: 283-287, 1945.
9. AHLQUIST, R. P. The interaction of sympathomimetic pressor amines. Inversion of the Vonedrine pressor action by Paredrine. J Am Pharm A (Scient Ed), 35: 348-350, 1946.
10. AHLQUIST, R. P. A study of the adrenotropic receptors. Am J Physiol, 153: 589-600, 1948.
11. AHLQUIST, R. P., HUOIGINA, R. A., AND WOODBURY, R. A. The pharmacology of benzyl imidazoline (Priscol). J Pharmacol & Exper Therap, 89: 271-283, 1947.
12. ALLEN, C. R., STRUTTMAN, J. W., STOCUM, H. C., AND ORTH, O. S. Protection from cyclopropane-epinephrine tachycardia by various drugs. Anesthesiology, 2: 503-514, 1941.
13. ALPERT, L. K., ALVINO, A. S., AND GRIMSON, K. S. Effect of total sympathectomy on experimental renal hypertension in dogs. Proc Soc. Exper Biol & Med, 37: 1-3, 1937.
14. AMELER, C. Über inverse Adrenalinwirkung. Arch f d ges Physiol, 185: 86-92, 1920.
15. ANAN, S. Über die Beziehungen zwischen den pharmakologischen Wirkungen und chemischen Konstitutionen von vielen chemischen Verbindungen der Reihe von Phenoxyäthylalkylamin. Japanese J M Sc., IV, 4: 70-71, 1929.
16. ANDERSON, L. H., AND WELLS, J. A. Attempts to prevent ergot gangrene with heparin and Dicumarol. Vascular effects of ergot by fluorescein technic. Proc Soc Exper Biol & Med, 67: 53-56, 1948.
17. BACQ, Z. M. Recherches sur la physiologie et la pharmacologie du système nerveux autonome. XXI. Nouvelles recherches sur les actions pharmacodynamiques des dérivés de l'aminométhylbenzodioxane (633F notamment),

- des amines à fonction éther-oxyde phénolique et de la yohimbine Arch. internat. de pharmacodyn. et de thérap., 52: 471-492, 1935
- 18 BACQ, Z. M., and BOVER, D. Recherches sur la physiologie et la pharmacologie du système nerveux autonome. XIII. Étude systématique des divers dérivés de l'aminométhylbenzodioxane et de l'aminométhylcoumarane, au point de vue de leur action sur les muscles lisses et le système nerveux sympathique. Arch. internat. de pharmacodyn. et de thérap. 50: 315-327, 1935
  - 19 BACQ, Z. M. and FREDERICO, H. Analyse de l'action sympatholytique du 833F (diéthylaminométhyl-3-benzodioxane) Compt. rend. Soc. de Biol., 118: 183-184, 1935
  - 20 BARBER, G. Ergot and ergotism Gurney and Jackson London, 1931
  - 21 BARGER, G. The alkaloids of ergot. In Hefter Handbuch der experimentellen Pharmakologie 6: 84-223 Julius Springer, Berlin, 1938
  - 22 BARGER, G., and DALE, H. H. Ergotamine and some other constituents of ergot. Biochem. J., 2: 240-299, 1907
  - 23 BARRETT, W., CAMERON, A., HANSEN, N., HERROLD, E., MACKENZIE, A., RICHARDS, B., ROTH, F., and SMITH, J. Toxicological and pharmacological data concerning 2-benzyl imidazoline hydrochloride (Priscol) Federation Proc., 7: 205, 1948
  - 24 BARRY, D. T. Some features of the pharmacological actions of yohimbine and ergotamine Arch. internat. de pharmacodyn. et de thérap. 55: 385-401, 1937
  - 25 BAUREHMEN, E. Die Kreislaufwirkung des Benzylimidazolins (Priscol) Arch. exper. Path. u. Pharmacol., 199: 161-166, 1942
  - 26 BEER, E. J. DE, and ULLAR, P. E. The biological estimation of ergometrine J. Pharmacol. & Exper. Therap. 71: 256-260, 1941
  - 27 BENNETT, G., and BOVER, D. Propriétés sympathomimétiques de la phénylméthanolhydrazine (1550 F) Compt. rend. Soc. de Biol., 136: 356-358, 1942
  - 28 BIELENDÜFEL, H. Akute Blutdrucksteigerung nach Priscol. München med. Wchnschr., 88: 588-589, 1941
  - 29 BING, R. J., and THOMAS, C. B. The effect of two dioxane derivatives 833 and 833 F, on normal dogs and on animals with neurogenic and renal hypertension. J. Pharmacol. & Exper. Therap., 83: 21-39, 1945
  - 30 BJORCK, G. Ergotamine and apparent coronary insufficiency. Brit. Heart J. 9: 181-184, 1947
  - 31 BJORCK, G. Studies on the action of dibenzyl- $\beta$ -chloroethylamine. Acta physiol. Scandinav., 14: 174-185, 1947
  - 32 BJORCK, G., and HALL, O. The effect of dibenzyl- $\beta$ -chloroethylamine on metabolism in rats. Acta physiol. Scandinav., 14: 186-188, 1947
  - 33 BLANCHER, M. Action, sur l'hyperglycémie adrénalinique du diéthylaminométhyl-3-benzodioxane (833F) et de quelques éthersoxydes phénoliques voisins. Compt. rend. Soc. de Biol., 115: 1185-1186, 1934
  - 34 BLUNTSCHLI, H. J. Clinical observations on the sympathetic inhibitory effects of a new ergot alkaloid. South African M. J., 21: 21-23, 1947
  - 35 BLUNTSCHLI, H. J. Über die Wirkung eines Ergot-Derivates (Dihydroergocornin) auf Blutdruck, Respiration und Elektrokardiogramm der Katz nach Querschnittsdurchtrennung des Rückenmarks im Bereich der Thoracal-Segmente. Helv. Physiol. et Pharmacol. Acta, 6: C50-C51, 1948
  - 36 BLUNTSCHLI, H. J. and GOETZ, R. H. The effect of a new sympatholytic drug (dihydroergocornine) on blood pressure with special reference to hypertension. South African M. J., 21: 352-401, 1947
  - 37 BLUNTSCHLI, H. J. and GOETZ, R. H. Ueber Kreislaufwirkungen neuer Mutterlorn Alkaloide am Menschen. Schweiz. med. Wchnschr., 77: 769-771, 1947
  - 38 BLUNTSCHLI, H. J., and GOETZ, R. H. The effect of ergot derivatives on the circulation in man with special reference to two new hydrogenated compounds (dihydroergotamine and dihydroergocornine). Am. Heart J., 35: 673-694, 1948
  - 39 BURN, J. DE, and DONOEN, K. VAN. Experiments with dihydroergotamine. Arch. internat. de pharmacodyn. et de thérap. 77: 434-441, 1948
  - 40 BOVER, D., and BOVER NITTI, F. Médicaments du système nerveux végétatif S. Karger, Basel, 1948
  - 41 BOVER, D., FOURNKAU, E., THÉROUILL, J., and STRICKLER, H. Le diéthylaminométhoxy-2-diphényle (1262 F) Propriétés pharmacologiques et activité sur la fibrillation du cœur. Arch. internat. de pharmacodyn. et de thérap., 62: 234-260, 1939
  - 42 BOVER, D., LESTRANGE, Y. DE, and FOURNKAU, J. P. Propriétés sympatholytiques de la N-phényl N isopropyl N-diéthyl éthylènediamine (1709 F) Compt. rend. Soc. de Biol., 136: 354-357, 1942
  - 43 BOVER, D. and MADER, J. P. Action sympatholytique de quelques amines à fonction éther-oxyde phénolique. Compt. rend. Soc. de Biol. 114: 980-983, 1933
  - 44 BOVER, D., and SIMON, A. L'action sur la musculature de l'iris de quelques substances sympatholytiques aminométhylbenzodioxanes (833 F et 933 F), yohimbine, corynanthine et corynantheine. Arch. internat. de pharmacodyn. et de thérap. 52: 413-433, 1935
  - 45 BOVER, D., and SIMON, A. Inversion de la tachycardie adrénalinique du lapin par le diéthylaminométhylbenzodioxane (833F), la corynanthine et la yohimbine. Compt. rend. Soc. de Biol., 119: 1333-1335, 1935
  - 46 BOVER, D., and SIMON, A. Le diéthylaminométhylbenzodioxane (833F) essais physiologiques sur les deux isomères optiques. Bull. d. sc. pharmacol., 42: 465-473, 1935
  - 47 BOVER, D. and SIMON, A. Recherches sur l'activité sympatholytique des dérivés de l'aminométhylbenzodioxane. Arch. internat. de pharmacodyn. et de thérap., 55: 15-31, 1937
  - 48 BOVER, D., and SIMON, A. Recherches sur la pharmacologie du système sympathique inhibiteur. 1. Activité de la méthoxy-2-ido-5-phenoxyéthyl-diéthylamine (1031F) Arch. internat. de pharmacodyn. et de thérap., 55: 223-232, 1937
  - 49 BOVER, D., SIMON, A., and DEPIERRE, F. Action centrale analgésique et sédatrice des aminométhylbenzo-

- dioxanes, des aminocoumaranes et des phénoxyéthylamines sympathicolytiques *Compt rend Soc. de biol.*, 117: 961-963, 1934
- 50 BOVER, D, SIMON, A ANN DRUY, J Propriétés pharmacodynamiques des oxyphénoxyéthylamines *Arch internat du pharmacodyn et de thérap*, 55 33 43, 1937
- 51 BOVER, D, TRÉFOUËL J, STERNÉ, J, ANN STRICKLER, H Action du diéthylaminodétoxy 2-diphényle (1263 F) sur la fibrillation cardiaque provoquée. *Compt rend Soc de biol*, 130 29-31, 1939
- 52 BRACK, W Ueber die Perniosis und die Raynaudsche Krankheit und ihre Behandlung mit Benzylimidazolin (Priscol) *Schweiz med Wchnschr*, 70 918-951, 1940
- 53 BRAUN MENÉNDEZ, E, PASCIOLLO, J C, LEMOIR, L F, MUÑOZ, J M ANN TAQUINI, A C Renal hypertension. Charles C Thomas, Springfield 1940
- 54 BROWN, G L, AND DALE, H The pharmacology of ergometrine. *Proc Roy Soc London*, s B, 118 416-477, 1935
- 55 BRÜGGER, J Die isolierte Samenblase des Meerschweinchens als biologisches Testobjekt zur quantitativen Differenzierung der sympathikolytischen Wirkung der genuine Mutterkornalkaloide und ihrer Dihydroderivate *Helv Physiol et Pharmacol Acta* 3 117-134 1945
- 56 BUCHANAN, A R ROBERTS, J E, ANN ROBINSON B E Ergotoxins hyper and hypothermia in albino rats. *Proc Soc. Exper Biol & Med* 68: 143-150 1948.
- 57 BUCHER, L, KOHLER, D ANN LÉVY J Relation entre la constitution chimique et l'action pharmacodynamique Création de substances antagonistes par doublement de la molécule *Bull Soc chim biol*, 29 788 793, 1947
58. BUCHER, L, ANN TCHOURAR, B Sur quelques sympatholytiques appartenant à la série des phényl thio-i diéthyl-amino-2-éthane *Compt Rend Soc de biol*, 141 34-35, 1947
- 59 BUCHER-OLASZYCKA, L Sur quelques nouvelles amines inversant l'action de l'adrénaline VI. Etude de leur action sur le coeur isolé de grenouille *Bull Soc chim biol*, 29: 547-553, 1947
- 60 BUCK, J S, ANN IDE, W S 2-alkyl 1,2 3 4 tetrahydroisoquinoline hydrochlorides *J Am Chem Soc.*, 60: 2101-2103 1938
- 61 BURN, J H, ANN DUTTA, N K The action of antagonists of acetylcholine on the vessels of the rabbit's ear *Brit J Pharmacol*, 3 351-361 1948
62. CACHERA, R Suppression par l'yohimbine du réflexe d'angiospasmе orthostatique chez l'Homme *Compt. rend Soc. de biol* 136 363-365, 1942
- 63 CAZEN, A Vitesse de disparition de l'adrénaline dans le sang Modifications apportées par l'injection préalable de substances inhibitrices (yohimbine, sympatholytiques de synthèse) *Compt rend Soc. de biol.*, 139: 24-26, 1945
- 64 CAHILL, G F Pheochromocytomas *J Am M A*, 138 180-186, 1948.
- 65 CANNON, W B, ANN LYMAN, H The depressor effect of adrenaline on arterial pressure *Am J Physiol*, 31: 376-398, 1913
- 66 CANNON, W B, ANN ROSENBLUTH A Studies on conditions of activity in endocrine organs XXIV. Sympathin E and sympathin I *Am J Physiol*, 104 557-574, 1933
- 67 CANNON, W B, ANN ROSENBLUTH, A Autonomic neuro-effector systems The Macmillan Company, New York, 1937
- 68 CHASE, H F, YONKMAN, F F, ANN LEHMAN, A J The effect of ethyl yohimbine in experimental hypertension *J Pharmacol & Exper Therap* 72: 6-7, 1941
- 69 CHASE, H F, YONKMAN, F F, ANN YOUNG A G Effect of certain yohimbine derivatives upon arterial strips *Proc. Soc Exper Biol & Med*, 40 308-310 1939
- 70 CHENS D ANN YONKMAN, F F A new adrenergic agent 2-benzyl imidazoline (Priscol) *Federation Proc*, 4 114 1945
- 71 CHENS D ANN YONKMAN, F F Adrenergic and sympatholytic actions of Priscol (benzyl imidazoline) *Proc Soc Exper Biol & Med*, 61 127-130 1948
- 72 CHEYMOU, J, ANN QUINQUAUD A Les poisons émetteurs devant l'yohimbine. *Compt. rend Soc. de biol*, 139 33-34 1945
- 73 CHEYMOU, J ANN QUINQUAUD, A Application d'ergotamine sur le bulbe émetteur. *Arch. internat. de pharmacodyn et de thérap* 77: 509-512, 1948
- 74 CHITTUM, J R, HILL, H C, JR., ANN GRIMSON, K. S An accoustical indicator of the systolic end point in rat blood pressure determinations *Proc. Soc Exper Biol & Med*, 66 486-488, 1947
- 75 CHRISTENSEN, L, SWAN, K C, ANN GOULD, J Hypotensive action of Dibenamine in glaucoma. *Northwest Med* 47: 731-732, 1948
- 76 CLARK, A J The antagonism of acetyl choline by atropine *J Physiol* 61: 547-556 1926
- 77 CLERC, A, STERNÉ, J, ANN LENOIR, J P Traitement des syndromes angineux par le 883F *Presse méd*, 46: 1553-1554 1938
- 78 CLYMER, N V, ANN SEUTER, J A method of screening sympathomimetic amines for stimulant action on the cerebrum *J Pharmacol & Exper Therap*, 89: 149-152 1947
- 79 CONSOLE, A D Diagnostic and therapeutic use of tetraethylammonium and Dibenamine *Am J Med* 5 184, 1948
- 80 COON J M, ANN SALERNO, P R A pharmacologic comparison of hexaethyl tetraphosphate (HETP) and tetraethyl pyrophosphate (TEPP) with physostigmine and neostigmine. *Federation Proc*, 7 212 213 1948.
- 81 CORET, I A Effects of a new congener of Dibenamine on the actions of sympathomimetic amines. *Proc Soc. Exper Biol & Med*, 68 553-558, 1948
82. CRAVER B N CHASE, H F, ANN YONKMAN, F F Pharmacologic studies of a new vasoconstrictor 2-naphthyl



- (17-methyl imidazoline hydrochloride (Privine or Naphazoline) II. Vascular and respiratory reactions in the anesthetized dog J Pharmacol & Exper Therap 82: 275-287 1944
- 83 DARIA, J Effets du pipéridométhyl benzodioxane (F 933) du diéthyl-méthyl amino-benzodioxane (F 833), du phénoxy-diméthylamino-éthane (L 407) et du méthoxy phénoxy-éthanol amino-éthane (L 418) sur l'utérus isolé Arch internat de pharmacodyn et de thérap 61 113-122 1939
  - 84 DARIA, J L'action du pipéridométhyl-3-benzodioxane (F 933) sur l'utérus in situ dépend du cycle sexuel Arch internat de pharmacodyn et de thérap, 63 359-375, 1939
  - 85 DALE, H H The physiological action of ergotamine J Physiol 32 LVII LX 1905
  - 86 DALE, H H On some physiological actions of ergot J Physiol 34, 103-206 1906
  - 87 DALE, H H On the action of ergotamine with special reference to the existence of sympathetic vasodilators J Physiol, 46 291-300 1913
  - 88 DALE, H H Nomenclature of fibers in autonomic system and their effects J Physiol, 80 10P-11P, 1934.
  - 89 DANIELOPOLU, D AND MARCOT, I. Action de l'adrénaline sur les coronaires et la circulation générale après les diéthylaminométhylbenzodioxane (833F) Compt rend Acad. d sc 206 692-694 1938.
  - 90 DANIELOPOLU D., POPESCU, M., AND MEZINCEAO, E Recherches sur l'action végétative des substances frénétiques (atropine, ergotamine, 833 F, yohimbine 2339 R P) Compt rend Soc sciobol 138 380-381, 1944.
  - 91 DANIELOPOLU, D., POPESCU, M., AND POPA, G G Action of drugs on cholinesterase and on the adrenolytic factors. Inactivating action of eserine, atropine and ascorbic acid on cholinesterase and on the adrenolytic factors. Acta Pharmacol et Toxicol 4 339-350 1948
  - 92 DAWES, G S Synthetic substitutes for quinidine Brit J Pharmacol, 1 90-112 1946
  - 93 DELAS AND STANISLAS Sur 3 hypotenseurs végétaux. Burus balcanica, Hedera helix, Gallium aparine Presse méd, 56 110 1948
  - 94 DEROUAUX, G Étude du mécanisme de l'action hémostatique de l'adrénaline Arch internat de pharmacodyn. et de thérap, 66 202-219, 1941
  - 95 DEROUAUX, G Action des poisons sympathicolytiques et adréno-lytiques sur le temps de saignement Arch. internat. de pharmacodyn et de thérap 66 231-240, 1941
  - 96 DONGEN, K. VAN The action of F 933 (Piperidomethyl-3-benzodioxane) on fibrillation of the heart Arch. internat. de pharmacodyn et de thérap 63 89-89, 1939
  - 97 DONGEN, K. VAN The action of diethylaminoethoxy 2-diphenyl (1282 F) on fibrillation of the heart. Arch. internat pharmacodyn. et de thérap, 66 41-44 1941
  - 98 DRAKE, M. E., AND THIESEN C H. The mydriatic effects of ergotamine tartrate on the normal and on the sympathetomized iris in normal and adrenalectomized rabbits Arch internat. de pharmacodyn. et de thérap, 65 390-392, 1941
  - 99 DRYER, N B AND HARWOOD, D  $\beta$ -10000 a sympatholytic agent Federation Proc, 6 324 1947
  - 100 DUMONT, L. Influence du 933F sur l'hypertension artérielle du chien en ischémie rénale chronique Compt. rend. Soc. de biol, 139 45-46, 1945
  - 101 DURANT, E., AND MAISON G Respiratory arrest in rabbits exposed to hypoxia after Dibenamine Proc Soc. Exper Biol & Med 67 435-437, 1948
  - 102 EISELE O Alkylamine derivatives U S Pat 1,949,247 Chem Abstr, 28 2350 1934
  - 103 ELDEN A. Pathophysiology of peptic ulcer A clinical study of 115 cases treated with ergotamine Acta med. Scandinav, 129 (Supp 202) 1-87, 1947
  - 104 EMERSON G A Effect of sympatholytic and ether agents on toxicity of digitalis in cats Proc Soc Exper Biol & Med. 53 12-14, 1943
  - 105 EMERSON G A Some circulatory effects of Privine hydrochloride J Pharmacol & Exper Therap, 82 42-47, 1944.
  - 106 EMERSON, G A., AND VAN LIEKE, E J Drug prophylaxis against lethal effects of severe anoxia. V Agents affecting the autonomic nervous system. J Lab & Clin Med. 28 700-708, 1943
  - 107 EULER, U S., VON Reflektorsche und zentrale Wirkung von Kaliumionen auf Blutdruck und Atmung. Skand. Arch f Physiol 80: 94-123 1938
  - 108 EULER, U S VON The presence of a sympathomimetic substance in extracts of mammalian heart J Physiol 105 38-44, 1946
  - 109 EULER, U S., VON Identification of the sympathomimetic ergone in adrenergic nerves of cattle (Sympathin N) with laevo-oadrenaline Acta Physiol. Scandinav, 16 63-74, 1948
  - 110 EULER, U S., VON AND HESLER, C M. Beobachtungen über Hemmungen der Sinusdruckreflexe durch Ergotamin, Dihydroergotamin und Hyperventilation. Schweiz med Wchnschr, 77 20-21 1947
  - 111 EULER, U S., VON AND LILJESTRAND, G The regulation of the blood pressure with special reference to muscular work. Acta physiol Scandinav, 12 279-300, 1946.
  - 112 EULER, U S., VON, AND SCHMITZLOW G G The action of ergotamine on the chemical and mechanical reflexes from the carotid sinus region Acta physiol. Scandinav, 8: 123-133 1944
  - 113 FARRETT D W AND HJORT, A. M. Some tetrahydroisoquinolines II. Their action on blood pressure, respiration and smooth muscle J Pharmacol & Exper Therap 63 253-271, 1938
  - 114 FOLKOW B FROST J., AND UYVÅS, B Action of adrenaline nor adrenaline and some other sympathomimetic drugs on the muscular cutaneous and splanchnic vessels of the cat Acta physiol Scandinav, 15 412-420, 1948.
  - 115 FOLKOW B, HAZEN, K AND UYVÅS, B Cholinergic vasodilator nerves in the sympathetic outflow to the muscles of the hind limbs of the cat Acta physiol Scandinav 15: 401-411 1948.
  - 116 FOLKOW B AND UYVÅS, B The chemical transmission of vasoconstrictor impulses to the hind limbs and the splanchnic region of the cat Acta physiol. Scandinav, 15: 365-383, 1948.

- 117 FOLKOW, B, AND UVNÄS, B The distribution and functional significance of sympathetic vasodilators to the hind limbs of the cat *Acta physiologica Scandinavica*, 15 389-400, 1948
- 118 FOURNEAU, E, AND BOVET, D Recherches sur l'action sympathicolytique d'un nouveau dérivé du dioxane. *Arch Internat de pharmacodyn et de therap*, 46 178-191, 1933
- 119 FREEMAN, N E, AND PAOZ, I H Hypertension produced by constriction of the renal artery in sympathetomized dogs *Am Heart J*, 14 403-414, 1937
- 120 FREEMAN, N E SHAFER, S A, SCHETTER, A E, AND HOLLING, H E The effect of total sympathectomy on the occurrence of shock from hemorrhage *J Clin Investigation*, 17 359-368, 1938.
- 121 FRIS, E D, STANTON, J R, AND WILKINS, R W The effects of certain dihydrogenated alkaloids of ergot in hypertensive patients *Am J M Sc*, 216 183-171, 1948
- 122 FRIS, E D, AND WILKINS, R W Effect of pentaquine in patients with hypertension. *Proc. Soc. Exper Biol & Med*, 64 455-458, 1947
- 123 FRIEDLAENDER, A S, AND FRIEDLAENDER, S An evaluation of Antistine, a new antihistaminic substance. *Ann. Allergy*, 6 23-29, 1948
- 124 FRIEDMAN, A P, AND BRENNER, C Treatment of the migraine attack *Am. Practitioner*, 2 467-470, 1948.
- 125 FRIEDMAN, M D, AND WILSON, E J Migraine Its treatment with dihydroergotamine (D.H.E. 45) *Ohio State M J*, 43 934-938, 1947
- 126 FRIEDMAN, O M, AND SELIGMAN, A M Derivatives of 10-methyl-1,2-benzanthracene related to the nitrogen and sulfur beta chloroethyl vesicants *J Am Chem Soc.*, 70: 3082-3086, 1948.
- 127 FROMMEL, E, BECK, I T, VALLETTE, F, AND FAVRE, M Études dans le domaine de la fièvre L'influence des substances sympatheco- et vagolytiques sur la fièvre diluée. *Helv Physiol et Pharmacol. Acta*, 5 376-381, 1947
- 128 FRUTON, J S, STEIN, W H, AND BERGMANN M Chemical reactions of the nitrogen mustard gases V The reactions of the nitrogen mustard gases with protein constituents *J Org Chem*, 11 559-570, 1948
- 129 FRUTON, J S, STEIN, W H, STAEMANN, M A, AND COLUMBIC, C Chemical reactions of the nitrogen mustard gases VI The reactions of the nitrogen mustard gases with chemical compounds of biological interest. *J Org Chem*, 11 571-580, 1946
- 130 FUGO, N W The antidiuretic action of yohimbine *Endocrinology*, 34: 143-148, 1944
- 131 FUGO, N W, AND GROSS, E G Anterior pituitary-stimulating action of yohimbine *Endocrinology*, 31: 529-534, 1942
- 132 GADNUM, J H, AND GOONWIN, L G Experiments on liver sympathin *J Physiol*, 105 357-369, 1947
- 133 GADNUM, J H, AND KWIATKOWSKI, H The action of ephedrine. *J Physiol*, 94: 87-100, 1938.
- 134 GANEM, E J, AND CAHILL, G F Pheochromocytomas coexisting in adrenal gland and retroperitoneal space, with sustained hypertension *New England J Med*, 238 692-697, 1948
- 135 GARB, S, AND CHENOWETH, M B Studies on hydrocarbon-epinephrine induced ventricular fibrillation *J Pharmacol & Exper Therap*, 94 12-18, 1948.
- 136 GERMAIN, J, AND BRUNAUD, M Action de l'adrénaline après le 933F sur l'intestin de rat *in vitro* *Compt. rend Soc de biol*, 138 842-844, 1944.
- 137 GERHARDT, B, AND ZOTTERMAN, Y The splanchnic efferent outflow of impulses in the light of ergotamine action. *Acta physiologica Scandinavica*, 11 301-317, 1946
- 138 GILBERT, N C Influence of extrinsic factors on the coronary flow and clinical course of heart disease *Bull New York Acad Med*, 18: 83-92, 1942
- 139 GILMAN, A, AND PHILLIPS, F S The biological actions and therapeutic applications of the  $\beta$ -chloroethyl amines and sulfides *Science*, 103 409-415, 1946.
- 140 GILES, M Über die Wirkungen eines neuartigen Imidazolins (Priscol-Ciba) auf das Auge *Arch f Ophth* 140 497-501, 1939
- 141 GLEY, E, AND CZARNIECKI, E L'apnée par yohimbine, inversion par l'adrénaline et, réciproquement inversion de l'apnée adrénalinique par la yohimbine *Compt rend Soc de biol*, 97 1159-1160, 1927
- 142 GOLDBLATT, H Renal origin of hypertension. *Physiol Rev*, 27: 120-165, 1947
- 143 GOLDENBERG, M, SNYDER, C H, AND ARANOW, E, JR New test for hypertension due to circulating epinephrine *J Am M A*, 135: 971-976, 1947
- 144 GOODMAN L S, WINTROBE, M M, DAMESCHKE, W, GOODMAN, M J, GILMAN, A, AND McLENNAN, M T Nitrogen mustard therapy *J Am M A*, 132: 126-132, 1946
- 145 GOWDEY, C W The change in pharmacological action produced by the introduction of a methyl group into Priscol *Brit J Pharmacol* 3: 254-262, 1948
- 146 GRAHAM, J R, AND WOLFF, H G Mechanism of migraine headache and action of ergotamine tartrate *Arch Neurol & Psychiat*, 39: 737-763, 1938.
- 147 GRANT, R L The effect of N N-dibenzyl- $\beta$ -chloroethylamine (Dibenamine) on blood sugar response to insulin and epinephrine *Federation Proc*, in press
- 148 GREENE L F The effects of drugs on ureteral activity *Proc. Staff Meet., Mayo Clin.*, 17: 404-410, 1942.
- 149 GREENGAARD H, COLLINS, C D, AND IVY, A C The effect of ergotamine on the external and internal secretions of the pancreas *Federation Proc.*, 4 25, 1945
- 150 GRIFFITH, J Q, AND COMROE, B I Reduced tolerance to ergotamine tartrate in hyperthyroidism. *J Pharmacol & Exper Therap* 69: 34-36, 1940
- 151 GRIMSON, K S, REARDON, M J MARXON, F A, AND HENDRIX, J P The effects of Priscol (2-benzyl-4,5-imidazoline HCl) on peripheral vascular diseases, hypertension and circulation in patients *Ann. Surg.*, 127: 988-990, 1948

152. GIMSON, K. S., WILSON, H., AND PREMISTER, D. B. The early and remote effects of total and partial paravertebral sympathectomy on blood pressure. *Ann Surg.*, 106: 801-825, 1937
153. GRINKEE, R. R., AND SPIVET, R. J. Ergotamine tartrate in the treatment of war neuroses. *J. Am. M. A.*, 127 158, 1945
154. GUNN, J. A. The pharmacological actions of morphothebaine-dimethylether. *J. Physiol.*, 100: 64-79 1941.
155. HAIMOVICI, H. Inhibitory effect of Dibenamine on vasoconstrictor substance. *Proc. Soc. Exper. Biol. & Med.*, 64: 488-488, 1947
156. HAIMOVICI, H. Evidence for an adrenergic component in the nervous mechanism of sweating in man. *Proc. Soc. Exper. Biol. & Med.*, 65 40-41, 1948.
157. HAIMOVICI, H. Postganglionic site of action of nicotine with special reference to its direct action on blood vessels. *Proc. Soc. Exper. Biol. & Med.*, 63 316-320, 1943.
158. HAIMOVICI, H., AND MEDINETS, H. E. Effect of Dibenamine on blood pressure in normotensive and hypertensive subjects. *Proc. Soc. Exper. Biol. & Med.*, 67 163-168, 1948
159. HAMBOURGER, W. E., AND JAMIESON, R. B. Comparison of the pressor effects of some new alkyl derivatives of  $\beta$ -phenylethylamine. *J. Pharmacol. & Exper. Therap.*, 58 33-61, 1938
160. HANDOVSKY, H. Les actions centrales paralyzantes provoquées par des substances sympathicolitiques. *Arch. internat. de pharmacodyn. et de therap.*, 75 449-452, 1948
161. HANSON, B. Action de la yohimbine sur les effets de l'adrénaline à l'égard du sucre du sang. *Compt. rend. Soc. de biol.*, 101: 603-605, 1929
162. HANZLIK, P. J., COTTING, W. C., HOBKINS, D., HANZLIK, H., BARNES, E. W., AND DORSETT, E. W. Vasomotor drugs on the convulsant threshold in rodents with and without diphenylhydantoin. *Stanford M. Bull.*, 6 47-53, 1948.
163. HARDGROVE, M., AND MENDELSON, D. J. Dibenamine. *J. Lab. & Clin. Med.*, 33 1496 1948
164. HARTMANN, M., AND ISLER, H. Chemische Konstitution und pharmakologische Wirksamkeit von in 2-Stellung substituierten Imidazolinen. *Arch. f. exper. Path. u. Pharmacol.*, 192 141-154 1939
165. HARTWELL, A. S., BURRETT, J. B., GRAYBIEL, A., AND WHITE, P. D. The effect of exercise and of four commonly used drugs on the normal human electrocardiogram with particular reference to T wave changes. *J. Clin. Investigation*, 21 409-417, 1942
166. HATT, F. Wirkung einiger neuer kreislaufaktiver Pharmaka (Priscol, Ottavin, Ciba 60E und Vadil) am Starling-schen Herz-Lungen Präparat. *Helv. Med. Acta*, 8 (Supp. 7) 70-79 1941
167. HAYES, D. W., WAKIM, K. G., HORTON, B. T., AND PETERS, G. A. The effects of dihydroergocornine on the peripheral circulation in man. *J. Lab. & Clin. Med.*, 33 1497, 1948
168. HAZARD, R., BEAUVALLET, M., AND GIUDICELLI, R. Action de l'yohimbine sur les effets cardio-vasculaires d'amino-alcools diphenoliques apparentés à l'adrénaline. *Compt. rend. Soc. de biol.*, 141 123-125 1947
169. HAZARD, R., BEAUVALLET, M., AND GIUDICELLI, R. Inversion par l'ergotamine des effets hypotenseurs exercés par deux amino-alcools diphenoliques apparentés à l'adrénaline. *Compt. rend. Soc. de biol.*, 141 591-592, 1947
170. HAZARD, R., BEAUVALLET, M., AND GIUDICELLI, R. Sur l'hypertension artérielle observée chez le chien après inversion, au moyen de l'ergotamine des effets normalement hypotenseurs de deux amino-alcools diphenoliques apparentés à l'adrénaline. *Compt. rend. Soc. de biol.*, 142 189-192 1948.
171. HAZARD, R., BEAUVALLET, M., AND GIUDICELLI, R. Recherches sur les modifications apportées par l'ergotamine aux effets vasculaires de l'isopropyladrénaline et de l'adrénaline. *Arch. internat. de pharmacodyn. et de therap.*, 77 504-508, 1948.
172. HAZARD, R. AND CHEYMOL, J. Effet d'excitation sympathomimétique produit sur le coeur du chien par le 933F. *Compt. rend. Soc. de biol.*, 135 1047-1050 1941
173. HAZARD, R., AND CHEYMOL, J. Action de l'ergotamine sur les effets vasculaires de la nicotine chez le chien surrénalectomisé. *Compt. rend. Soc. de biol.*, 136 350-351 1942
174. HAZARD, R. AND CHEYMOL, J. Action de l'yohimbine et du 933F sur les effets vasculaires de la nicotine chez le chien surrénalectomisé. *Compt. rend. Soc. de biol.*, 136 397-398 1942
175. HAZARD, R., CHEYMOL, J., AND QUINQUAUD, A. Non inversion de l'apnée adrénalinique par l'yohimbine chez le lapin. *Compt. rend. Soc. de biol.*, 128 529-532, 1938.
176. HAZARD, R., CHEYMOL, J., AND QUINQUAUD, A. Inversion par l'yohimbine, de l'apnée ergotaminique. *Compt. rend. Soc. de biol.*, 135 253-254 1941
177. HAZARD, R., FRESCALINE, Y., AND JÉQUIER, R. Sur l'hyperglycémie provoquée chez le lapin par le 933 et le 853F. *Compt. rend. Soc. de biol.*, 135 1091-1094 1941
178. HAZARD, R. AND MOISSET DE ESPANES, E. Action du pipéridométhyl-3-benzodioxane (933 F) sur l'intestin isolé de cabaye. *Arch. internat. de pharmacodyn. et de therap.*, 59 457-460, 1938.
179. HAZARD, R., AND QUINQUAUD, A. Yohimbine sympathicolitique et potassium vasoconstricteur. *Compt. rend. Soc. de biol.*, 136 439-440 1942
180. HAZARD, R., AND VAILLE, C. Action de l'adrénaline sur l'intestin isolé du rat en présence du pipéridométhyl-3-benzodioxane (933 F). *Compt. rend. Soc. de biol.*, 137 715-717, 1943
181. HEATH, R. G. AND POWDERMAKERS, F. The use of ergotamine tartrate as a remedy for "battle reaction." *J. Am. M. A.*, 125 111-113, 1944.
182. HEBB, C. O. Bronchomotor responses to stimulation of the stellate ganglia and to injection of acetylcholine in isolated perfused guinea pig lungs. *J. Physiol.*, 99 57-75, 1940
183. HECUT, H. H. AND ANDERSON, R. B. The influence of Dibenamine (N,N-dibenzyl  $\beta$ -chloroethyl amine) on certain functions of the sympathetic nervous system in man. *Am. J. Med.*, 3 3-17 1947
184. HENDRIX, J. P., REARDON, M. J., AND MARZONI, F. A. Observations on the effects of benzyl imidazoline (priscol) in man. *Federation Proc.*, 6 338, 1947

- 185 HERMANN, H, JOURDAN, F, AND BONNET V Action sympatholytique et adrénalinoinversive du 2-benzyl imidazoline Compt rend Soc de biol, 135: 1653-1655, 1941
- 186 HERMANN, H, JOURDAN, F, MORIN, G, AND VIAL, J Effets tensionnels de l'injection intraveineuse continue d'adrénaline chez le chien dioxané Compt rend Soc de biol, 131: 282-284, 1939
- 187 HERMANN, H, AND VIAL J Sur quelques actions pharmacodynamiques du 2-benzyl imidazoline (C 3259) Compt rend Soc de biol, 136: 803-805, 1942
- 188 HERWICK, R P, LINEGAR, C R, AND KOPFANYI, T Factors controlling vasomotor reversal to epinephrine J Pharmacol & Exper Therap, 63 15, 1933.
- 189 HERWICK, R P, LINEGAR, C R, AND KOPFANYI, T The effect of anesthesia on the vasomotor reversal J Pharmacol & Exper Therap, 65 185-190, 1939
- 190 HESS, W R Neur u psych Abh aus Schw Arch Neur u Psych, 2 3, 1925 Quoted in STOLL, A Experimentis, 1: 250-262, 1945
- 191 HEYMANS, C, AND BOUCKAERT, J J Au sujet de l'action vasomotrice et vasculaire de l'ergotamine. Arch. Internat pharmacodyn et de therap, 46: 129-136, 1933
- 192 HEYMANS, C, AND BOUCKAERT, J J Au sujet des influences du pipéridométhyl-3 benzodioxane (F 933) sur le système circulatoire. Compt rend Soc de biol, 120: 79-82, 1935
- 193 HEYMANS, C, BOUCKAERT, J J, ELAUT, L, BAYLESS, F, AND RAMAAN, A Hypertension artérielle chronique par ischémie rénale chez le chien totalement sympathéctomisé Compt rend Soc. de biol, 126 43-45, 1937
- 194 HILL, W T, AND MYERS, G S The effect of pipéridométhyl-3-benzodioxane (933 F) on the heart of the frog and the cat Am J Physiol, 126 305-309, 1939
- 195 HJORT, A M, BEER, E J NE, BUCK, J S, AND RANDALL, L O Relative pharmacological effects of 2-alkyl-1,2 3 4 tetrahydroisoquinoline hydrochlorides J Pharmacol & Exper Therap, 76: 64-70, 1942
- 196 HJORT, A M, BEER E J NE, AND FASSETT, D W Some tetrahydroisoquinolines. IV The circulatory adrenolytic effects J Pharmacol & Exper Therap, 68 73-79, 1940
- 197 HUNT, C C Structural relationship to sympatholytic activity of certain  $\beta$ -chloroethyl amines Federation Proc., 7: 229, 1948
- 198 HUTCHINSON, M, SHAW, F H, AND WBAOGE, W B A contribution to the pharmacology of yohimbine. Australian J Exper Biol & M Sc, 20: 69-71, 1942
- 199 IMPFELD, J P Erste klinische Erfahrungen mit Dihydroergotamin (D H E 45) Schweiz. med. Wchnschr., 76 1263-1266, 1946
- 200 JACOBS, J, AND YONKMAN, F F Sympatholytic treatment of experimental hypertension J Lab & Clin. Med, 29 1217-1221, 1944
- 201 JACOBSON, L O, SPURN, C L, BARRON, E S G, SMITH, T, LUSHBAUGH, C, AND DICK, G F Nitrogen mustard therapy J Am M A, 132: 263-271 1946
- 202 JAFFE, N S Sympathetic nervous system and intraocular pressure Am J Ophth, 31 1597-1603, 1948.
- 203 JANO C S Interaction of sympathomimetic substances on adrenergic transmission J Pharmacol & Exper Therap, 70: 347-361, 1940
- 204 JANO, C S The potentiation and paralysis of adrenergic effects by ergotoxins and other substances J Pharmacol & Exper Therap, 71: 87-94, 1941
- 205 JANOT, M M, BOVET D, AND MONTHEIN, G Propriétés sympatholytiques des dérivés acétylés de la corynanthine Compt rend Soc de biol, 137: 278-279, 1943
- 206 JOURDAN, F Action du F 883 et du F 933 sur la pression artérielle du chien sans moelle Compt. rend. Soc. de biol, 119: 1258-1260, 1935
- 207 JOURDAN, F, AND FROMENT, R Influence de quelques sympatholytiques sur le rythme lido-ventriculaire. Compt rend Soc de biol., 130: 1264-1268, 1939
- 208 JORDAN, F, AND GUILLET, P Mécanisme de l'action des benzodioxanes chez le chien chloralé Compt. rend. Soc de biol, 136: 807-809, 1942
- 209 JUSTIN BESANÇON, L, BOVET, D, AND KOHLER, D Action anti-exophtalmique et myotique de la corynanthine Compt. rend Soc. de biol, 118: 532-534, 1935
- 210 KAHANE, E, AND LÉVY, J I Sur quelques nouvelles amines inversant l'action de l'adrénaline Bull Soc chim biol, 27 562-570 1945
- 211 KARR, N W Circulatory and adrenolytic actions of DHE and DHO Federation Proc., 7: 332 1948
- 212 KATZ, L N, AND FRIENBERG L The hemodynamic effect of the dioxane derivative 933 F on trained unanesthetized normal and renal hypertensive dogs and its effect on the pressor action of renin Am J Physiol, 127 29-36, 1939
- 213 KATZ, L N, AND LINDBER, E The reaction of the coronary vessels to drugs and other substances J Am M A 113: 2116-2118, 1939
- 214 KELLEY D M The use of ergotamine compounds in the treatment of acute simple anxiety states. Am J Psychiat, 104: 608-612, 1948
- 215 KEOOH P AND SHAW, F H The pharmacology and toxicity of Alstonia alkaloids Australian J Exper Biol & M Sc. 21 163-188, 1943
- 216 KHARASCH, M S, KINO, H, STOLL, A, AND THOMPSON, M R The new ergot alkaloid. Science, 83: 206-207, 1936
- 217 KING T O A comparative study of sympatholytic drugs Federation Proc., 6: 345 1947
- 218 KIRCHHOFF, A C, RACKLEY, C A, WILSON, W M, AND DAVID N A An ergonovine-like oxytocic synthesized from lysergic acid West J Surg, 52: 197-208 1944
- 219 KOHLER, D AND LÉVY, J Sur quelques nouvelles amines inversant l'action de l'adrénaline III. Influence

- de ces substances sur les réactions de l'intestin vis à vis de l'adrénaline et de l'éphédrine Bull. Soc. chim. biol., 28 121-125 1948
- 220 KUHLE D. AND LÉVY J. Sur quelques nouvelles amines inversant l'action de l'adrénaline. IV Influence de quelques amines à fonction éther-oxyde sur l'action excito-intestinale de l'acétylcholine Bull. Soc. chim. biol. 28 349-352, 1946
- 221 KUHLE, D., AND LÉVY J. Sur quelques nouvelles amines inversant l'action de l'adrénaline. V Influence de quelques amines à fonction éther-oxyde sur l'action excito-intestinale du chlorure de baryum Bull. Soc. chim. biol. 28 333-334, 1946
- 222 KOLM R. AND PICK, E. P. Über die Bedeutung des Calciums für die Erregbarkeit der sympathischen Herznervenendigungen Arch. f. d. ges. Physiol. 189 137-143 1921
- 223 KOPET J. C., AND DILL, J. M. The elimination of ergotamine, ergotamine and ergonovine J. Am. Pharm. A. (Scient. Ed.) 31 109-113, 1942
- 224 KOPFANT, T. A new artificial ergot derivative dehydroergotamine (D.H.E. 45) Anat. Rec. 96 584, 1947
- 225 KOPFANT, T., LINERAR, C. R., AND HERWICK, R. P. The vasomotor reversal in yohimbine-treated dogs Federation Proc. 2 83-84 1943
- 226 KOTZE, D. C. BOBB J. R., AND GREEN H. D. Vasomotor activity of NU-1683 and other drugs upon the rat meso-appendix. Federation Proc. 7 235 1948
- 227 KUTSCHERA AICHBERGER H. Diuresemmittel Wien klin. Wchnschr. 57 93-94 1944
- 228 LAURIN, E. Ergotoxin and adrenalinhyperglykämie Biochem. Ztschr. 82 87-95, 1917
- 229 LENSEL, R. The secondary blood pressure rise and tachycardia occurring after the injection of epinephrine Federation Proc. 7 71 1948
- 230 LENOGENHABER H. Beobachtungen an Raynaud Patienten. Schweiz. med. Wchnschr. 77 97-98, 1947
- 231 LENOX W. G. Ergonovine versus ergotamine as a terminator of migraine headaches Am. J. M. Sc., 1951; 433-463, 1939
- 232 LEROY G. V. NALEFSKI, L. A. AND CHRISTY H. W. The effect of certain sympatholytic agents on the coronary blood flow of the dog. J. Lab. & Clin. Med., 33 1493 1948.
- 233 LÉVY J., AND DITZ, E. Sur quelques amines à fonction éther oxyde phénolique inversant certaines actions pharmacodynamiques de l'adrénaline. Arch. internat. de pharmacodyn. et de therap., 47 138-268, 1934
- 234 LÉVY J. AND THIBAUT, C. Sur quelques nouvelles amines inversant l'action de l'adrénaline II. Etude de leur action sur les bronches Bull. Soc. chim. biol., 28 114-120 1946
- 235 LEWIS H. A., AND GOLDBLATT H. Studies on experimental hypertension. Bull. New York Acad. Med., 18 45-167 1942.
- 236 LILJESTRAND A. Interaction of ergotamine and carbon dioxide on blood pressure and respiration Acta physiol. Scandinau., 15 198-206, 1943
- 237 LINDBLUM, T. Die Behandlung der Raynauischen Krankheit mit 2-Benzyl-4-5-imidazolin (Priscol Ciba) Acta med. Scandinau., 113 83-108, 1943
- 238 LINERAR, C. R. The modification of the hemodynamic effects of acetylcholine by ergotamine Am. J. Physiol., 129: 53-68, 1940
- 239 LISA, K. The influence of piperidinomethylbenzodioxane (933 F) on the action of sympathin. Am. J. Physiol., 124 62-65, 1938
- 240 LIEW, E. R. Pharmacology of antihistamine compounds Physiol. Rev., 27 542-573 1947
- 241 LIEW, E. R. AND MICETICH, A. Adrenergic blocking drugs. I. Comparisons of effectiveness in decreasing epinephrine toxicity in mice J. Pharmacol. & Exper. Therap., 93 434-443 1948.
- 242 LIEW, E. R., MICETICH, A., AND ACHENBACH, P. Antagonism of the excitatory responses to epinephrine and adrenergic nerve stimulation with benzhydryl alkyl- $\beta$ -chloroethyl amines. Federation Proc. 6 351-352 1947
- 243 LIEW, L. AND LENKE S. E. The use of estrogenic hormone in experimental peripheral gangrene J. Pharmacol. & Exper. Therap. 63 93-98 1938
- 244 LIEW, S. Die Wirkung synthetischer Cycloethylamine aus der Verwandtschaft von Adrenalin und Histamin auf autonome Erfolgsorgane Ztschr. f. d. ges. exper. Med. 56 271-333 1927
- 245 LIEW, S. Influence of autonomic drugs on ejaculation. J. Pharmacol. & Exper. Therap. 63 70-75 1938.
- 246 LIEW, S. Personal communication
- 247 LIEW, S. Über eine postganglionäre Wirkung von Nikotin und Acetylcholin. J. Physiol. USSR 22: 390-394, 1937
- 248 MACHT D. I. On the pharmacology of the ureter. I. Action of epinephrin, ergotoxin and of nicotine. J. Pharmacol. & Exper. Therap. 8 155-168 1916
- 249 MACNEAL, P. S. AND DAVIS D. The use of methyl iso-octenylamine in migraine Ann. Int. Med., 26: 526-527, 1947
- 250 MADDOCK, W. O., RANKIN V. M. AND INUMANA, W. B. Prevention of the anti-cure action of epinephrine by Dibensamine. Proc. Soc. Exper. Biol. & Med. 67 151-153 1948
- 251 MALORNY G. AND ORZECZAKOWSKI, G. Untersuchungen über die Wirkungsweise der Sympathicomimetica. II. Vergleichende Untersuchungen über die Beeinflussung der Adrenalinwirkung durch ephedrinähnlich gebaute Substanzen. Arch. f. Exper. Path. u. Pharmacol. 196 245-252 1940
- 252 MANVINO C. W. AND CANNWELL, G. C. The effect of Demerol, ergotamine, and dihydroergotamine on mortality after coronary occlusion in dogs Brit. Heart J. 9 35 95, 1947
- 253 MARSH D. F. AND VAN LIERE, E. J. The effect of adrenergic blocking agents on the vasoconstriction produced by acute oxygen lack J. Pharmacol. & Exper. Therap. 94 221-224, 1943
- 254 MARTIN J. AND LISA, K. Einfluss des Piperidinomethylbenzodioxane (933 F) auf die Adrenalinreaktion des isolierten Säugerherzens. Arch. f. Exper. Path. u. Pharmacol. 196: 1-5 1940.

- 254 MARZONI, F A, REARDON, M J AND HENDRIX, J P The effects of benzyl imidazoline (Priscol) in partially sympathectomized dogs. *Federation Proc*, 6: 355, 1947
- 255 MARZONI F A, REARDON, M J HENDRIX, J P, AND GRIMSON, K S Effects of Priscol, tetra-ethyl ammonium ion, and Dibenamine on certain vascular reflexes and peripheral resistance in dogs and man. *Bull. Am. College Surgeons*, 32 232, 1947
- 256 MASTER, A M, PONDY, L, AND KOLKER, J Ergotamine tartrate and the "two-step" exercise electrocardiogram in functional cardiac disturbance. *J Mt. Sinai Hosp*, 15 164-168, 1948
- 257 McALLISTER, F F, AND ROOT, W S The circulatory responses of normal and sympathectomized dogs to ether anesthesia. *Am J Physiol*, 133: 70-78, 1941
- 258 MEDINETS, H E, KLINE, N S, AND METTLER F A Effects of N,N-dibenzyl- $\beta$ -chloroethylamine hydrochloride (Dibenamine) on autonomic functions and catatonias in schizophrenic subjects. *Proc. Soc. Exper Biol & Med*, 69: 238-246, 1948
- 259 MEIER, R The differentiation of sympathicotropic drug action. *Schweiz med. Wchnschr* 76: 880-885, 1946.
- 260 MEIER, R., AND BUCHER, K Kreislaufwirkung des Pravina und Adrenalins, ihre antagonistische Beeinflussbarkeit durch "Sympatholytika" *Helv Physiol Pharmacol Acta*, 4: 69-76, 1946
- 261 MEIER, R., AND MEYER, R T Ueber den peripheren Angriffspunkt des Priscols am Gefäßsystem. *Schweiz. med Wchnschr*, 71 1206-1207, 1941
- 262 MEIER R., AND MÜLLER R Gefäßwirkung eines neuen Imidazolin Derivates. *Schweiz med Wchnschr*, 69 1271-1275 1939
- 263 MEIER, R AND YONKMAN, F F Personal communication
- 264 MELVILLE, K I The antisymphathomimetic action of dioxane compounds (F833 and F933), with special reference to the vascular responses to dihydroxyphenyl ethanolamine (arterenol) and nerve stimulation. *J Pharmacol. & Exper Therap*, 39 317-327, 1937
- 265 MENDEZ R Antagonism of adrenaline by ergotamine. *J Pharmacol & Exper Therap*, 32 451-464, 1928.
- 266 MEYER, R T Ueber die 'adrenalinolytische' Wirkung des Priscols (Benzylimidazolin) an den Gefäßen der isolierten Hinterextremität des Kaninchens. *Helv Medica Acta*, (Supp 7), 8 18-24, 1941
- 267 MINZ, B, AND PETERFALVI, M Sur un aspect nouveau de l'action biologique des sympatholytiques. *Compt. rend Acad d sc*, 224: 1589-1591 1947
- 268 MINZ, B, AND VEIL, C Rigidité de décharge et sympatholytiques. *Compt rend Soc de biol.*, 141. 573-574, 1947
- 269 MOE, G M, MALTON, S D, RENNICK, B R AND FREYBURGER, W A The role of arterial pressure in the induction of idioventricular rhythms under cyclopropane anesthesia. *J Pharmacol & Exper Therap*, 94: 319-327, 1948
- 270 MOE, G K., AND SEEVERS, M H Central impairment of sympathetic reflexes by plasmochin. *Federation Proc*, 5 193-194, 1946
- 271 MOIR, C The action of ergot preparations on the puerperal uterus. *Brit. M J*, 1 1110-1123, 1933
- 272 MÖLLER, E Vergiftung mit Vasodil (Priscol). *Kasuist. Meddelelser. Nord Med*, 33 610-612, 1947
- 273 MOORE, R M, AND CANNON, W B The heart rate of unanesthetized normal, vagotomized, and sympathectomized cats as affected by atropine and ergotoxine. *Am J Physiol*, 94: 201-208, 1930
- 274 MORIN, G Sur l'action adréno lytique du pipéridométhyl-3-benzodioxane (933 F) à l'égard de l'intestin isolé du lapin. *Compt rend Soc de biol*, 130 1267-1269, 1939
- 275 MORISON, R S, AND LISSAK, K Observations on the mode of action of piperidinomethylbenzodioxane (933 F). *Am J Physiol*, 123 404-411, 1938
- 276 MÜLLER, R Vergleichende Pharmakologie des Priscol. *Schweiz med Wchnschr* 75 29-34, 1945
- 277 NANDA, T C The action of ergotamine on the response of the rabbit's gut to adrenaline. *J Pharmacol & Exper Therap*, 42 9-16, 1931
- 278 NABO, J A new test for gastro function. *Am J Digest Dis*, 11 227-239, 1944
- 279 NICKERSON M Chemical adrenergic blockade. *Proc. First Nat Med Chem. Symp*, (ACS) 70-80, 1948
- 280 NICKERSON M Interpretation of experimental results obtained with Dibenamine. *Endocrinology*, in press
- 281 NICKERSON, M Unpublished results
- 282 NICKERSON, M, BERGHOUT, J, AND HAMMERSTROM R. N Mechanism of the acute lethal action of epinephrine in rats. *Federation Proc*, in press
- 283 NICKERSON, M BROWN, H O, AND SMITH, S M The mechanism and efficacy of Dibenamine protection against "spontaneous" cyclopropane arrhythmias in surgical patients. *Am J Med.*, in press.
- 284 NICKERSON, M, BULLOCK, F AND NOMAOUCHI, G M Effect of Dibenamine on renal hypertension in rats. *Proc. Soc. Exper Biol & Med*, 68 425-429, 1948.
- 285 NICKERSON, M, AND GOODMAN, L S The pharmacology of a series of new sympatholytic agents. *Proc. Am Federation Clin. Research* 2 109-110 1945
- 286 NICKERSON, M, AND GOODMAN, L S Pharmacological properties of a new adrenergic blocking agent N,N-dibenzyl- $\beta$ -chloroethylamine (Dibenamine). *J Pharmacol & Exper Therap*, 89: 167-185, 1947
- 287 NICKERSON, M, AND GOODMAN, L S Pharmacological and physiological aspects of adrenergic blockade, with special reference to Dibenamine. *Federation Proc*, 7 397-409, 1948.
- 288 NICKERSON M, AND GUMP, W S The chemical basis for adrenergic blocking activity in compounds related to Dibenamine. To be published.
- 289 NICKERSON, M, AND GUNN, F D Unpublished results
- 290 NICKERSON, M, AND HARRIS, F B Antihistaminic properties of the  $\beta$ -haloalkylamines (Dibenamine) series of adrenergic blocking agents. *Federation Proc.* in press.

- 291 NICKERSON M AND HARRIS, F B Unpublished results
- 292 NICKERSON, M, AND NOMAGUCHI, G M Locus of the adrenergic blocking action of Dibenamine J Pharmacol. & Exper Therap, 93 40-51 1948.
- 293 NICKERSON, M, AND NOMAGUCHI, G M Mechanism of Dibenamine protection against cyclopropane-epinephrine cardiac arrhythmias J Pharmacol. & Exper Therap, 95 1-11 1949
- 294 NICKERSON, M, AND NOMAGUCHI, G M The depressor activity of sympathomimetic amines in the presence of Dibenamine blockade. To be published
- 295 NICKERSON M, AND NOMAGUCHI, G M Unpublished results
- 296 NICKERSON M, NOMAGUCHI, G, AND GOONMAN L S. Relation of structure to activity in a new series of sympatholytic agents Federation Proc, 5 195, 1948
- 297 NICKERSON, M, AND PRICE, P B Unpublished results
- 298 NICKERSON, M, AND SMITH, S M Protection against cyclopropane-epinephrine arrhythmias by Dibenamine and other agents. Anesthesiology in press
- 299 NICKERSON, M, SMITH, S M, AND GOONMAN, L S The prevention of epinephrine-cyclopropane cardiac irregularities in dogs with dibenzyl- $\beta$ -chloroethyl amine. Federation Proc., 5 195-198, 1948.
- 300 NICKERSON, M AND WANG C Y Unpublished results
- 301 NORDENFELT, O Über funktionelle Veränderungen der P- und T Zaken im Elektrokardiogramm, experimentelle Untersuchungen mit Ergotamine und Amyl nitrit, sowie klinische Beobachtungen. Acta Med Scandinav, Sup 119 1-158f 1941
- 302 OGDEN, E The extra renal sequel to experimental renal hypertension. Bull New York Acad Med, 23 643-660, 1947
- 303 OGDEN, E. Influence of dibenamine on renal function of the dog Federation Proc, 7 87 1948
- 304 ORTH O S Studies on the sympathicolytic drug Dihydroxyergotamine (D.H.E. 45) Federation Proc. 5 198, 1948
- 305 ORTH, O S, AND RITCHIE, G A pharmacological evaluation of dihydroergotamine methanesulfonate (D H E 45) J Pharmacol. & Exper Therap, 90 164-173, 1947
- 306 PAGE I. H. Special aspects of the problem of the renal origin of hypertension. Bull New York Acad Med. 19 461-477, 1943
- 307 PARIJS, R, JANOT, M M AND GOUTARIEL R Sur quelques propriétés physiologiques et en particulier sur l'action sympatholytique de la corynanthéidine Compt rend Soc. de biol. 139 665-666, 1945
- 308 PLANELLER, J Mutterkornstudien. I. Über das Zusammenwirken von Ergotamin und Adrenalin am Meerschweinchen Darm. Arch. f exper Path u Pharmacol, 105 38-48, 1923
- 309 POHLE, F AND SÄRRE, H Der Wirkungsmechanismus eines neuen peripheren Kreislaufmittels im Vergleich mit Veritol und Sympatol beim Menschen. Arch. f exper Path u Pharmacol., 196 408-430, 1940
- 310 PRITTKER, B Die Beeinflussung der psychomotorischen Erregung durch Dihydroergotamin (DHE 45) Schweiz. med. Wchnschr, 77 935-987 1947
- 311 RAAB, W The pathogenic significance of adrenalin and related substances in the heart muscle. Exper Med & Surg, 1 188-225, 1943
- 312 RAAB, W, AND HUMPHREYS, R J Protective effect of adrenolytic drugs against fatal myocardial epinephrine concentrations J Pharmacol. & Exper Therap, 58 263-278, 1948
- 313 RAAB W AND HUMPHREYS, R J Drug action upon myocardial epinephrine-sympathin concentration and heart rate (nitroglycerine, papaverine Prisol, Dibenamine Hydrochloride) J Pharmacol. & Exper Therap, 89 64-76, 1947
- 314 RAYMOND-HAMET Sur un nouveau cas d'inversion des effets adrénaliniques Compt rend. Acad. d sc., 180: 2074-2077, 1925
- 315 RAYMOND-HAMET Sur l'inversion de l'action normale de l'adrénaline. Compt rend. Acad. d. sc, 183 485-486, 1926
- 316 RAYMOND-HAMET Sur un nouveau sympathicolytique vrai La Rauwolfine de Koepfl. Compt. rend. Acad d sc 201 1050-1053 1935
- 317 RAYMOND-HAMET Sur le pouvoir anesthésique local de la corynanthine et sur l'inversion, par cet alcaloïde, des effets respiratoires de l'adrénaline Compt rend. Soc. de biol., 118 774-777 1935
- 318 RAYMOND-HAMET La bulbocapnine, type d'un nouveau groupe de médicaments. Compt rend. Acad. d sc 202 357-359, 1936
- 319 RAYMOND-HAMET Sur une nouvelle méthode de mise en évidence du pouvoir sympathicolytique Compt rend. Acad d. sc., 203: 208-210, 1936.
- 320 RAYMOND-HAMET Sur quelques propriétés physiologiques de la boldine Compt. rend. Soc. de biol., 121 431-434, 1936.
- 321 RAYMOND-HAMET Différence du comportement physiologique des sympathicolytiques vrais du groupe de l'ergotoxine et de ceux du type de la yohimbine Compt. rend Soc de biol, 122 1277-1279, 1936.
- 322 RAYMOND-HAMET Über die Wirkung von Corynanthin auf die männliche Genitalfunktion. Arch. f exper Path. u Pharmacol, 184 650-655, 1937
- 323 RAYMOND-HAMET Sur les effets hypotenseurs et sympathicolytiques majeurs de l'hydrocinnonidine Compt. rend. Acad. d sc. 207 1252-1254 1935
- 324 RAYMOND-HAMET Sur une curieuse propriété physiologique de l'extrait aqueux de Rauwolfia heterophylla Roem et Sch. Compt. rend. Acad. d sc. 209 384-388, 1939
- 325 RAYMOND-HAMET Sur un nouveau faux Iboga pharmacologiquement actif Compt. rend. Acad d sc., 210-783-791, 1940

- 326 RAYMOND-HAMET Sur une propriété insoupçonnée de la pyridine Compt rend Soc de biol, 136 781-783 1942.
- 327 RAYMOND-HAMET La diacétylation de la yohimbine et de la corynanthine modifie-t-elle l'action sympathicolytique de ces alcaloïdes? Compt rend Acad d sc 216 611-616, 1943
- 328 RAYMOND-HAMET Modifications de l'activité physiologique de la yohimbine résultant de sa transformation en base ammonium quaternaire Compt rend Acad d sc 217 181-183 1943
- 329 RAYMOND-HAMET Production d'un nouveau type de poisons du système nerveux sympathique par iodométhylation d'un sympathicolytique Compt rend Acad d sc, 218 425-427, 1944
- 330 REED R K, SAPIRSTEIN L A, SOUTHWARD, F D, JR AND OGDEN, E The effects of Nembutal and yohimbine on chronic renal hypertension in the rat Am J Physiol 141 707-712, 1944
- 331 REMINGTON J W, WHEELER N C, BOYD, G H JR, AND CADRELL, H M Protective action of Dibenamine after hemorrhage and after muscle trauma Proc Soc Exper Biol & Med, 69 150-151 1948
- 332 RHOADE, C P Nitrogen mustards in the treatment of neoplastic diseases J Am M A, 131 658-658 1948.
- 333 RICHARDS B, CAMERON, A, CRAVER, B AND HERHOLD E Detoxification of 2-benzyl imidazole (Prisol) by incubation with hepatic slices from the rat Federation Proc 7 251, 1948
- 334 RICHARDSON A P, WALKER H A, AND MILLER, B S Effect of pentamine on cardiovascular system of an anesthetized dog Proc Soc Exper Biol & Med, 65 258-261 1947
- 335 RICHARDSON J A, AND WALTON, R P Further analysis of the influence of autonomic innervation on drug responses of the heart and gut in unanesthetized dogs Federation Proc 7 251 1948.
- 336 ROCH M, AND ROCH BESSER B De l'utilité du chlorhydrate de Benzyl Imidazole (Prisol) dans les affections cérébrales consécutives à des déficits circulatoires Schweiz med Wchnschr, 77: 679-680, 1947
- 337 ROCKWELL F V Dibenamine therapy in certain psychopathologic syndromes Psychosom Med, 10: 230-237 1948
- 338 ROSENBLUTH A, AND ACHESON, G H Does the nictitating membrane of the cat have a refractory period? Am J Physiol, 120 514-521, 1937
- 339 ROSENBLUTH, A, AND CANNON, B Some circulatory phenomena disclosed by ergotoxins Am J Physiol, 105 373-382, 1933
- 340 ROSS, J F The effect of pipendinomethylbenzodioxane (833F) and yohimbine upon the action of certain drugs and ions on the nictitating membrane Am J Physiol, 116: 574-576, 1938
- 341 ROSS, J F Is the transmission of the nerve impulses to the cat's uterus electrical or chemical? Am J Physiol, 119 527-530, 1937
- 342 ROTHLIN E Recherches expérimentales sur l'Ergotamine, alcaloïde spécifique de l'Ergot de Seigle Arch Internat pharmacodyn et de thérap, 27 459-470, 1923
- 343 ROTHLIN E Über die pharmakologische und therapeutische Wirkung des Ergotamins auf den Sympathicus Klin Wchnschr 4 1437-1443 1925
- 344 ROTHLIN, E The specific action of ergot alkaloids on the sympathetic nervous system J Pharmacol & Exper Therap, 36 657-683, 1929
- 345 ROTHLIN E Ueber ein neues Mutterkornalkaloid Schweiz med Wchnschr, 65: 947-949, 1935
- 346 ROTHLIN E Über die Standardisierung von Mutterkorn Arch f exper Peth u Pharmacol 184: 69-71 1936.
- 347 ROTHLIN, E Sur les propriétés pharmacologiques d'un nouvel alcaloïde de l'ergot de seigle, l'ergobasine. Compt. rend Soc de biol, 119 1302-1304, 1938
- 348 ROTHLIN, E Zur Pharmakologie der hydrierten natürlichen Mutterkornalkaloide Helv Physiol et Pharmacol Acta., 2. C48-C49, 1944
- 349 ROTHLIN E Ueber die adrenergischen Funktionen am Herz und an Gefässen Bull schweiz Akad med Wissensch, 1: 194-206, 1945
- 350 ROTHLIN, E Zur Pharmakologie des Sympathicolyticums Dihydroergotamin DHE 45 Schweiz med. Wchnschr, 76 1254-1259, 1946
- 351 ROTHLIN E The pharmacology of the natural and dihydrogenated alkaloids of ergot Bull Schweiz. Akad med Wissensch, 2 249-273, 1947
- 352 ROTHLIN, E Ueber das Schicksal der natürlichen und dihydrierten Mutterkornalkaloide im Organismus Schweiz med. Wchnschr, 77: 1161-1163 1947
- 353 ROTHLIN E, AND BRÜGGER, J Quantitative Untersuchungen der sympathikolytischen Wirkung gegenüber Mutterkornalkaloide und deren Dihydroderivate am isolierten Uterus des Kaninchens Helv Physiol. et Pharmacol Acta, 3 519-535, 1945
- 354 ROTHLIN, E AND RAYMOND-HAMET Sur la toxicité et l'activité adrénolytique de la pseudocorynanthine comparées avec celles de la corynanthine et de la yohimbine Arch Internat de pharmacodyn et de thérap, 50: 241-250 1935
- 355 SACHS E AND YONKMAN, F F The pharmacological behavior of the intracocular muscles V The action of yohimbine and ergotamine on the dilator iridis J Pharmacol & Exper Therap, 75 105-110 1942
- 356 SALANT, W, AND PARKINS, W M The effect of ergotamine on the intestine with observations on the influence of calcium on its action J Pharmacol & Exper Therap, 44 369-383, 1932
- 357 SAPIRSTEIN, L A., AND REED, R K Effects of Fournau 833 and Fournau 933 on late renal hypertension in the rat Proc Soc Exper Biol & Med 57: 135-136, 1944
- 358 SAWYER C H, MARKEE J E, AND HOLLINGSHEAD W H Inhibition of ovulation in the rabbit by the adrenergic blocking agent Dibenamine. Endocrinology, 41 395-402 1947
- 359 SAWYER, M E M AND SCHLOSSBERG, T Studies of homeostasis in normal sympathetomized and ergotaminized animals I The effect of high and low temperatures Am J Physiol 164: 173-183 1933
- 360 SCHAEPDEJUYER, A DE Actions pharmacologiques sur le muscle bronchique des poumons isolés du cobaye normal et anaphylactisé Arch internat. de pharmacodyn et de thérap 77 46-47, 1918.



- 361 SCHÄR WÖTHMICH, B Die Wirkungen einiger kreislaufaktiver Substanzen auf Fermente. *Helv Chim. Acta*, 26 1836-1835, 1943
- 362 SCHERF, D, AND SCHLACHMAN, M. The changes in the electrocardiogram associated with standing. *Proc Soc. Exper Biol. & Med.*, 68: 150-153, 1948
- 363 SCHMETZ, H., AND FLUCH, M. Priscol, ein neues gefäßerweiterndes Mittel. *Ztschr f klin. Med.*, 137: 667-684, 1940
- 364 SCHMETZ, H., AND FLUCH, M. Über den Einfluss von Priscol auf die Sekretion der Magendrösen. *Ztschr f klin. Med.*, 140 593-603, 1942
- 365 SKIFFES, J. Personal communication
- 366 SHAW, F H, KEOGH, P, AND MACCULLUM, M. The possibility of the dual nature of sympathetic ganglion cells. *Australian J Exper Biol. & M. Sc.*, 24 139-146, 1948.
- 367 SHEN, T C R. Influences du pipérido-méthyl benzodioxane (F 933) sur le cœur de grenouille. *Compt rend. Soc de biol.*, 124. 274-276 1938.
- 368 SHEN, T C R. Influence of pipérido-méthyl-3-benzodioxane (F 933) upon the beat of isolated frog's heart. *Arch. internat de pharmacodyn. et de thérap.*, 55 493-501, 1937
- 369 SHEN, T C R. Dissociation of the pupil-dilating action of adrenaline from that of sympathetic stimulation by means of pipérido-méthyl-3-benzodioxane, diethyl amino-méthyl-3-benzodioxane and yohimbine. *Arch. internat. de pharmacodyn. et de thérap.*, 58 484-491, 1938.
- 370 SHEN, T C R. The protective action of pipérido-méthyl-3-benzodioxane (F 933), diethyl amino-méthyl-3-benzodioxane (F 883) and yohimbine upon the chloroform adrenaline ventricular fibrillation. *Arch. internat. pharmacodyn. et de thérap.*, 59 243-251, 1938.
- 371 SHEN, T C R. The mechanism of the melanophore-expanding action of several drugs and its relationship to the internal secretion of the hypophysis in frogs. *Arch. internat de pharmacodyn. et de thérap.*, 62: 295-329, 1939
- 372 SHEN, T C R., AND MARRI, R. Further studies on cardio-ventricular fibrillation. A. Influence of diethyl-aminoethoxy-2-diphenyl (F 1262), corynanthine and p-oryphenyl-ethanol methyl amine (sympathol) on the benzol adrenaline cardio-ventricular fibrillation in the dog. B. The role of the hypertensive action of adrenaline. *Arch. internat. de pharmacodyn. et de thérap.*, 64. 58-78, 1940
- 373 SIMONE, F A., AND SARXOFF, S J. The effect of Dibenzamine on autonomic stimulation. *Surgery*, 22 391-401, 1947
- 374 SIVADIAN, J. L'action du diéthylaminométhyl-3-benzodioxane (883F) et de la corynanthine sur la fièvre provoquée par les agents chimiques. *Compt. rend. Soc de Biol.*, 114 568-570 1933
- 375 SOLLMANN T., AND BROWN, E D. Intravenous injection of ergot. Effects on the mammalian circulation. *J Am M A.*, 45 229-240 1905
- 376 SPEAR H C, AND GRISWOLD, D. The use of Dibenzamine in pheochromocytoma. *New England J Med.*, 239 738-739, 1948.
- 377 SPÖHLER, O. Dihydroergotamin (DHE 45) als Sympathicolyticum in der inneren Medizin. *Schweiz. med. Wchnschr* 76 1239-1263 1948.
- 378 SPÖHLER, O. Die experimentelle Untersuchung eines neuen Sympathicolyticums, des Dihydroergotamins (DHE 45). *Schweiz. med. Wchnschr* 77 23-32 1947
- 379 SPÖHLER, O. Neue Wege in der medikamentösen Therapie der Sympathicus-Erkrankungen. *Praxis, Schweiz. Rundschau f Med.*, 37 557-560, 1948
- 380 STAHMANN, M A., FRUTON, J S, AND BERGMANN, M. Chemical reactions of mustard gas and related compounds. VI. The chemistry of sulfonium salts related to mustard gas. *J Org. Chem* 11 704-718, 1946
381. STAHMANN, M A., GOLUMBI, C, STEIN, W H, AND FRUTON J S. Chemical reactions of mustard gas and related compounds. VII. The chemistry of bis( $\beta$ -chloroethyl)-sulfone, divinyl sulfone and divinyl sulfoxide. *J Org. Chem* 11 719-735, 1946.
382. STERNÉ, J., BOYER, D., AND LENDIN, J. Effects du diéthylaminométhylbenzodioxan (883F) sur les lésions myocardiques provoquées. *Compt. rend. Soc de biol* 139 207-209, 1939
- 383 STOLL, A. Les alcaloïdes de l'ergot. *Experientia*, 1 250-262, 1945
384. STOLL, A. AND BURCKHARDT, E. Ergocristin und Ergocristinin, ein neues Alkaloidpaar aus Mutterkorn. *Zeit. f Physiol Chem.*, 250 1-8 1937
- 385 STOLL, A., AND HOFMANN A. Partialsynthese von Alkaloiden von Typus des Ergobaains. *Helv Chim. Acta*, 26 944-965, 1943
386. STOLL, A., AND HOFMANN A. Die Alkaloide der Ergotoxingruppe. Ergocristin, Ergokryptin und Ergocornin. *Helv Chim. Acta*, 26: 1570-1601, 1943
- 387 STOLL, A., AND HOFMANN, A. Die Dihydroderivate der natürlichen linksdrehenden Mutterkornalkaloide. *Helv Chim. Acta*, 26 2070-2081 1943
388. STOLL, A., HOFMANN, A., AND BECKER, B. Die Spaltstücke von Ergocristin, Ergokryptin und Ergocornin. *Helv Chim. Acta*, 26 1602-1613 1943
- 389 STOLL, W A. Lysergäure-diäthylamid, ein Phantasticum aus der Mutterkorngruppe. *Schweiz. Arch. f Neurol. n. Psychiat.*, 69 1-45, 1947
- 390 STONE, C A., ACHENBACH, P, AND LOEW, E R. Adrenergic blocking properties of certain halogenated ethylamine derivatives. *Federation Proc.*, 7 253, 1948.
- 391 SULMAN, F, AND BLACK, R. The alleged endocrine effect of yohimbine. *Endocrinology*, 36 70-72 1945
- 392 SWANSON, E E., AND CHEN, K. K. Comparison of pressor action of alicyclic derivatives of aliphatic amines. *J Pharmacol. & Exper Therap.*, 93 423-429, 1948.
- 393 TEAGUE, R. S., NOOTH, R O, AND GILLING, E M. K. The hypophysectomized frog (*Rana pipiens*) as a specific test object for melanophore hormone of the pituitary body. *J Pharmacol. & Exper Therap.*, 65: 115-127 1940

- 394 TEPPERMAN, J, AND BOOARDUE, J S Attempts at pharmacologic blockade of the secretion of adrenocorticotrophin *Endocrinology*, 43 418-450 1948.
- 395 THIELE, W Präcol ein neues Mittel für die Magendiagnostik *Klin Wchnschr* 19 520-521 1940
- 396 THIENES, C H Action of ergot alkaloids on intestine and uterus *Proc Soc. Exper Biol & Med*, 26: 501-502 1929
- 397 THOMPSON, R. M, AND COON, J M Effect of adrenergic agents on the response to pressor substances in the domestic fowl *Federation Proc*, 7 250 250 1948
- 398 TILLOREN, N Treatment of headache with dihydroergotamine tartrate *Acta med. Scandinav*, Supp 196 222-228, 1947
- 399 TORDA, C The effect of cocaine, ergotamine and yohimbine on the activity of phenol sulfatase *J Pharmacol & Exper Therap*, 77: 123-126, 1943
- 400 TORDA, C, AND WOLFF, H G Experimental studies on headache Transient thickening of the walls of cranial arteries in relation to certain phenomena of migraine headache and action of ergotamine tartrate on thickened vessels *Arch Neurol & Psychiat*, 53 329-332, 1945
- 401 TOURNANE, A, CHARDON, G, AND CALLÉJA, A Le phénomène de Philippeaux Vulpian Effects paralyzants de l'yohimbine. *Compt. rend Soc de biol*, 135 383-384, 1941
- 402 TOURNANE, A, CHARDON, G, AND CALLÉJA, A Système vaso-moteur et yohimbine Action de l'alcaloïde sur les récepteurs sinu-carotidiens *Compt. rend Soc de biol*, 135 446-448, 1941
- 403 TOURNANE, A, CHARDON, G, AND CALLÉJA, A Système vaso-moteur et yohimbine. Action de l'alcaloïde sur les centres vaso-moteurs *Compt. rend Soc. de biol*, 135 544-545, 1941
- 404 UJVÁŠ, B Action of n n-dibenzyl chloroethylamine (Dibenamine) on the effect of sympathetic secretory impulses to the submaxillary gland of the cat. *Acta physiol Scandinav*, 15: 362-364, 1948
- 405 VAN LIERE, E J, NORTHUP, D W, AND STRICKNEY, J C The influence of agents affecting the autonomic nervous system on the motility of the small intestine. *Am. J Physiol*, 141 462-465, 1944
- 406 VERNER, E B, AND VOOT, M An experimental investigation into hypertension of renal origin, with some observations on convulsive "uraemia" *Quart. J Exper Physiol* 28 253-303, 1938.
- 407 VLEESCHOUWER, G DE Au sujet de l'action du diéthylaminométhyl-3 benzodioxane (F 883) et du pipéridométhyl-3-benzodioxane (F 933) sur le système circulatoire *Arch internat de pharmacodyn et de therap*, 50 251-295, 1935
- 408 VLEESCHOUWER, G R. de Pharmacology of dibenzyl  $\beta$ -chloroethylamine hydrochloride (Dibenamine) *Proc. Soc. Exper Biol & Med*, 66: 151-152, 1947
- 409 VLEESCHOUWER, G R. de On new adrenergic compounds *Proc Soc. Exper Biol & Med*, 67 298-299, 1948.
- 410 WAXIM, K G, AND CHEN, K. K The action of alstonine. *J Pharmacol & Exper Therap*, 90: 57-67, 1947
- 411 WANO, C Y, AND NICKERSON, M Effect of Dibenamine on renal function. *Proc. Soc. Exper Biol & Med*, 70 92-96, 1949
- 412 WEITZMANN, G Zur Behandlung der peripheren Durchblutungsstörung mit Präcol *München. med. Wchnschr*, 88: 99-102, 1941
- 413 WELLS, J A, MORRIS, H C, BULL, H B, AND DRAGSTENT, C A Observations on the nature of the antagonism of histamine by  $\beta$ -dimethylaminoethyl benzhydrol ether (Benadryl) *J Pharmacol & Exper Therap*, 85 122-128, 1945
- 414 WELLS, J A, AND RALL, D P The influence of N (2-bromoethyl)-N-ethyl 1 naphthalene-methylamine on the vasopressor response of a series of amines *Federation Proc*, 7: 364, 1948
- 415 WELLS, J A, AND RALL, D P Personal communication
- 416 WENKHOE, M H The influence of autonomic imbalance on the human electrocardiogram I. Unstable T waves in precordial leads from emotionally unstable persons without organic heart disease. *Am Heart J*, 28: 549-567, 1944
- 417 WENKHOE, M H Supplemental use of ergotamine in eliciting transient disturbances of rhythm in acute rheumatic fever *Proc. Soc. Exper Biol & Med*, 58 303-306, 1945
- 418 WENKHOE, M H, AND LONIE, R B Unstable T waves in leads II and III in persons with neurocirculatory asthenia. *Am Heart J*, 31: 711-723, 1945
- 419 WEST, G B Quantitative studies of adrenaline and noradrenaline *J Physiol*, 106: 418-425, 1947
- 420 WHITE, A C A comparison of some of the ergot alkaloids. Part I General toxicology *Quart J Pharm & Pharmacol*, 16: 344-352 1943
- 421 WIKZUCHOWSKI, M, AND BIELINSKI, Z Action du pipéridométhyl-3-benzodioxane (F 933) sur les neuro-récepteurs du sinus carotidien *Compt rend Soc. de biol*, 131 269-270, 1939
- 422 WIONER, H C, INGRAHAM, R. C, RHEIMOLD, F, AND GLENBERG, H Vasoconstriction and the development of irreversible hemorrhagic shock *Am J Physiol*, 153 511-520, 1948.
- 423 WILBURNE, M, KATZ, L N, ROBBARD, S AND SURTSHIN, A The action of N,N dibenzyl beta-chloroethylamine (Dibenamine) in hypertensive dogs *J Pharmacol & Exper Therap*, 90 215-223 1947
- 424 WUNSCH, R E, WARNEKE, R D, AND MYERS, G B The effects of Dibenamine in severe hypertension. *Ann Int. Med*, in press
- 425 YATER, W M, AND CAHILL, J A Bilateral gangrene of feet due to ergotamine tartrate used for pruritus of jaundice *J Am M A*, 106 1625-1631, 1936
- 426 YONKMAN, F F Ergotamine miosis *J Pharmacol & Exper Therap*, 43 251-261 1931
- 427 YONKMAN, F F Clinical application of sympatholytic agents *Proc. First Nat. Med Chem Symp (ACS)*, pp 81-86, 1948

- 428 YONKMAN, F F, HAYS, H. W., CAMERON, A., PELLET, E., AND HANSEN, N. Cholinergic action of the anti-sympathetic agent priscol (benzylmefedazoline HCl) *Federation Proc.*, 5: 216, 1946.
- 429 YONKMAN, F F, JEREMIAS, R., AND STILWELL, D. Angiotonin myotropism. *Proc. Soc. Exper Biol. & Med.*, 54: 204-206, 1943.
- 430 YONKMAN, F F, STILWELL, D., AND JEREMIAS, R. The adrenolytic and sympatholytic actions of yohimbine and ethyl yohimbine. *J Pharmacol. & Exper Therap.*, 81: 111-115, 1944.
- 431 YONKMAN, F F, AND YOUNG, A. G. Further studies on yohimbine derivatives. *J Pharmacol. & Exper Therap.*, 57: 151, 1935.
- 432 YOUNG, W B, AND RANKIN, V M. Effects of Dibenamine on cardiovascular actions of epinephrine, acetylcholine, pitressin and angiotonin in unanesthetized dogs. *Proc. Soc. Exper Biol. & Med.*, 66: 241-244, 1947.
- 433 YOUNG, A. G. Chemotherapeutic studies of yohimbine compounds. *J Pharmacol. & Exper. Therap.*, 54: 164-165, 1935.
- 434 ZUNZ, E., AND JOURDAN, F. Recherches a propos de l'action du diéthylaminométhyl-3-benzodioxane et de composés voisins sur la diurèse par l'eau, par la solution chlorurée sodique et par l'urée. *Arch. internat. de pharmacodyn. et de therap.*, 48: 383-428, 1934.
- 435 ZUNZ, E., AND VESSELOVSKY, O. Effets des combinaisons moléculaires d'alcoïdoles de l'ergot sur la diurèse. Ergoclavine, ergosine et ergosinine. *Compt. rend. Soc. de biol.*, 128: 1165-1167, 1938.
- 436 ZUNZ, E., AND VESSELOVSKY, O. Action de l'ergocristine et de l'ergocristine sur la diurèse. *Compt. rend. Soc. de biol.*, 129: 20-22, 1938.
- 437 ZUNZ, E., AND VESSELOVSKY, O. Action de la corynanthine et de la yohimbine sur la diurèse aqueuse. *Compt. rend. Soc. de biol.*, 131: 135-138, 1939.
- 438 ZWAHLEN, P. Le Priscol dans les brûlures oculaires. *Schweiz. Med. Wchnschr.*, 77: 858, 1947.

# THE INTERACTIONS OF DRUGS AND PLASMA PROTEINS\*

AVRAM GOLDSTEIN

*From the Department of Pharmacology, Harvard Medical School, Boston*

**INTRODUCTION** The capacity of drug molecules to enter into specific combinations with proteins poses for pharmacology its most fundamental task—to comprehend the intimate nature of drug action in terms of these molecular interactions. The great contributions of Clark and of Ehrlich, in their time, shifted the focus from organism to cell. But modern advances in enzymology, protein chemistry, microbiology and electron microscopy have made the cell appear gross and complex. Modern pharmacology, of necessity, has also come to reckon in Ångström units and to approach its problems at the level where drug molecules exert their peculiar effects upon the molecular structure and function of living protoplasm.

Most drugs have been established, or can be surmised, to act by virtue of combination with specialized functional proteins<sup>1</sup>. To these proteins the vague term "receptors" has been assigned in the past, but their precise natures and roles are finally being clarified. Ehrlich (79, 80), on the basis of his observations of dye affinity for tissue proteins and the remarkable specificities of antibody-antigen reactions, proposed that chemical combination of drugs with tissue constituents was the *sine qua non* of pharmacological action. "*Corpora non agunt nisi fixata*". As he had hoped, the field of chemotherapy now provides most eloquent confirmation of this concept. The "chemoreceptors" of parasitic organisms prove to be protein enzymes, vital links in a metabolic chain, exquisitely sensitive to chemotherapeutic agents of precise specificity. Thus the mode of action of all chemotherapeutic agents is being investigated currently at the molecular level. In the field of general pharmacology the cholinesterase inhibitors are outstanding examples of drugs whose actions are now understood in terms of specific interaction with a protein enzyme playing a special role in certain highly localized regions. The vitamins, on the other hand, function as prosthetic groups to widely distributed enzymes. Heparin owes its characteristic effect to interference with a complex system of interacting proteins in the plasma and the evidence points toward a specific interaction with one or more of these proteins. Can it be seriously doubted that the barbiturates, the salicylates, morphine and its congeners, the cardiac glycosides, the curare compounds, the local anesthetics, the autonomic drugs will also prove to enter into definite specialized relationships with particular tissue proteins?

Curiously, the specificity of drug-protein interactions is often more apparent than real. Drugs are in fact capable of forming a multitude of alliances with

\* Original investigations and other work incident to the preparation of this review were generously supported by grants from the Division of Research Grants and Fellowships of the National Institute of Health, United States Public Health Service, and the Abbott Laboratories, North Chicago, Illinois.

<sup>1</sup> There are certain obvious, if trivial, exceptions, e.g., saline cathartics and diuretics.

the proteins of the body, some reversible and transient, others firm and persistent. The key fits many locks but there is only one door! That single combination which is responsible for the "primary drug action" forces itself upon our attention. If certain other interactions also lead to obvious physiological changes we note that a "side effect" has occurred, and if such changes are detrimental we apply the term "toxic effect." But the great bulk of drug molecules may enter into "silent" combinations which, from the standpoint of therapeutic effect, seem irrelevant. An example is the fixation of quinacrine to the liver, where its concentration may be a thousand times that in the parasitized erythrocytes which are the sites of therapeutic action (163).

Among these numerous secondary interactions are those in which the plasma proteins participate. Are these in any sense unique? Bennhold (11), whose many accomplishments in this field will be reviewed in later sections, was led by his observations to the view that the plasma proteins constitute a specially designed transport mechanism for the regulated distribution of naturally occurring and medicinal substances throughout the body. "Blut ist die flüssige Transportform des Gewebes im tierischen Organismus." Whether one accepts the teleological overtones of Bennhold's formulations one must recognize that the plasma proteins are indeed endowed with remarkable reactivity, that associations of the most varied kind occur with the most diverse of chemical substances, and that the sojourn of such molecules in the body may be profoundly influenced by these interactions. Whether the proteins of other tissues are equally versatile in their reactivities cannot be established at present. Indeed, the difficulties inherent in studying interactions with the proteins of fixed tissues present a sharp contrast to the readiness with which those of the plasma lend themselves to investigation. Furthermore, the remarkable progress of recent years in the isolation and characterization of plasma proteins has provided considerable impetus to the study of these rather than other interactions. A major influence in this direction has been the large scale program of plasma fractionation through which investigators have been able to obtain standardized human plasma protein preparations of known homogeneity (47, 48).

Consequently a resurgence of interest in drug-protein interactions is apparent among pharmacologists today, further stimulated perhaps by the demonstration that in the clinical use of the sulfonamides and penicillins these phenomena are of some practical importance. Interactions have, in fact, been the subject of perennial investigation, for over half a century, by pharmacologists, physiologists and chemists alike. But much of this work, in its application to the living organism, was purely empirical in concept and execution and inconclusive in result. Lacking a clearly formulated theoretical orientation, it led as often to greater confusion as to greater clarity.

It will be the aim of this review not only to present a factual summary of some of the most significant studies on interactions but also to set forth the author's concept of a rational approach to the problem from the standpoint of pharmacology. A systematic collection of the pertinent data obtained in numerous interaction studies will be presented in tabular form, but the work of various

investigators will be cited in the text only when it bears directly upon the topic under discussion. There will also be no attempt to exclude interacting compounds other than drugs when their consideration seems appropriate.

**METHODS OF DEMONSTRATING INTERACTION** At least sixteen different methods have been employed to demonstrate the presence of an interaction between drug and protein molecules but these rest upon only three basic principles.

(1) The concentration of free drug and its thermodynamic activity are reduced, and its biological action may also be diminished. Whether such effects are measurable depend upon their magnitude, and upon the sensitivity of the methods employed.

(2) The drug may show changed properties which can be measured with greater or less precision. Some of these effects may also be attributed to reduction of thermodynamic activity.

(3) The protein component may be measurably altered with respect to its properties or behavior.

The several methods based upon each of these principles will be discussed, showing for each the kind of results that can be obtained, the conditions which must be observed, and the conclusions that can safely be drawn.

#### 1 *Methods based upon reduction in free drug concentration*

a *Biological action* The observation that serum is capable of interfering with the action of many drugs has often served as initial stimulus to the study of drug-protein interactions. Busck (33), as early as 1906, reported on the inhibitory effect of serum on the photodynamic action and other toxic effects of certain dyes, using paramecia as test organisms. He showed that serum not only interferes with dye action but also alters diffusibility, fluorescence or light absorption, solubility, and other properties. These phenomena he attributed to the formation of dye-albumin complexes.

Storm van Leeuwen and his collaborators (245, 246) discovered that rabbit serum inhibits the action of pilocarpine and atropine on the isolated gut of the cat, serum alone produced no effect. The alkaloids are not destroyed, as evidenced by their complete recovery on alcohol denaturation of the serum proteins.

Reduction of biological activity on the frog heart was used by McLean and Hastings (169) (after the technique introduced by Trendelenburg (263)) as the basis of their classical quantitation of the serum binding of calcium. A similar quantitative analysis is embodied in the studies of Fawaz and Farah (86), and of Farah (85), on cardiac glycosides, using systolic arrest of the frog heart for the assay of free drug concentration in the presence and absence of various serum protein fractions.

More recently the inhibitory effect of albumin on the hemolytic action of fatty acids has been employed as an investigative tool by Boyer, Ballou and Luck (24). Davis and Dubos (56, 58) studied the abolition by albumin of the antibacterial action of oleic acid on the tubercle bacillus, and the binding of diverse chemotherapeutic agents has been examined in similar fashion.

This method is fundamentally sound from the pharmacological standpoint.

provided the drug concentrations employed are within the range to be expected *in vivo*, and provided the protein concentration has not been artificially altered (e.g., by dilution) If these provisions have been met one can then expect to detect an interaction if an appreciable fraction of the total drug is bound to protein at the concentration investigated As bioassay methods are crude at best it is probably safe to assume that unless the unbound drug concentration is reduced by one-quarter to one-half, reliable results will not be obtained. Furthermore the conditions must be such that the binding is not disturbed by the determination itself For example, if a cardiac glycoside were three-quarters bound to plasma protein in a loose, reversible combination, and the affinity of the drug for cardiac muscle were greater than for plasma protein, all the drug might be rapidly removed from combination and it would appear that none had been bound Again, in the bioassay of penicillin, preliminary dilution in a protein-free solution results in rapid dissociation of the bound drug so that the original binding is no longer evident in the assay Therefore while reduction of biological effect as a criterion of interaction may yield extremely useful information, especially if positive results are obtained, interpretations must be extremely cautious when "no binding" is found The possibility of direct effect of the protein upon the biological assay material must, of course, be ruled out.

b *Dialysis* Confining the protein component within a semi-permeable membrane through which unbound drug molecules can freely diffuse is one of the oldest and most direct methods of demonstrating interaction (184) At equilibrium the unbound drug activities on both sides of the membrane must be equal and any increment in the protein compartment is presumed to represent bound drug Outstanding recent applications of this technique are the studies of Klotz (138, 140, 141) on the binding of anionic dyes and purine derivatives Provided the membrane (usually cellophane sausage casing) is proved to be impermeable to protein and permeable to the drug in question, and that equilibrium is actually attained within the period of the experiment, the accuracy of the method is determined wholly by that of the procedure for ascertaining the drug concentration This need not require simultaneous determinations in the aqueous and protein solutions for a method of differences can be employed, on the assumption that the observed loss of drug from the protein-free dialysate represents drug in combination with protein (138) However, inaccuracies may result from irregular adsorption to the dialysis bag itself In the author's experience with methylene blue, such loss may not only be large (up to 20% of the total dye at low concentrations) but variable from bag to bag The Donnan effect must also be considered and appropriate corrections made in the case of ionic drugs, especially when the total ionic strength is low Indeed the same corrections apply to all methods wherein a portion of the aqueous phase is separated from the protein regardless of the presence of a physical membrane

Bendien and Snapper (9, 10) employed a technique of differential dialysis, the pores being of such size that albumin molecules passed through but larger proteins did not This method is unwieldy and unpredictable, and offers no real advantage over other currently available techniques

Dialysis is eminently suited for quantitative studies, especially when an inter-

action is to be explored through a range of drug concentrations. In this way nearly complete saturation of a protein with a given drug can often be achieved and information of prime physico-chemical importance derived. It is one of the few methods conducive to such quantitative work and it is thermodynamically sound. On the other hand it does not yield accurate information about the status of an interaction in the plasma *in vivo*. Even if whole plasma containing the substance under study is dialyzed, physiological conditions will no longer obtain because of the dilution of the water phase. If the drug-protein combination is reversible, dissociation may occur and neither the amount bound per mole protein nor the fraction of total drug bound will necessarily reflect the state of affairs before the equilibrium was disturbed.

c *Ultrafiltration*. This method (6, 8) differs from dialysis in that the water phase is expressed through a semipermeable membrane by pressure and there is consequently no dilution of the system. The technique is appealing in its simplicity and various types of apparatus have been described (51, 147, 159, 234). It probably approximates most closely the process of glomerular filtration since the hydrostatic pressures employed are usually of a magnitude comparable to the renal filtration pressure.

Bennhold (11) suggests that the lability of a drug-protein bond is somehow connected with ultrafiltrability, that bonds which are not disrupted under milder conditions (electrophoresis, diffusion) may be broken by the pressure under which ultrafiltration is carried out. In support of this view he cites instances of apparently complete binding in diffusion and electrophoresis experiments of substances which nevertheless appear in an ultrafiltrate. If this view were correct ultrafiltration would yield little valid information about reversible reactions for it presumes that equilibria can be shifted by the conditions of filtration. However, from the thermodynamic standpoint the argument is palpably implausible. An equilibrium can be altered in favor of diminished binding only by reducing the *concentration* of a reactant or increasing the *dissociation constant* of the complex. Expression of the water phase with its dissolved drug does not accomplish the former, and the latter, depending as it does upon the rate at which molecules attach to and detach from the protein surface, could hardly be affected significantly by the filtration pressure.

For precise quantitative studies the method is inferior to dialysis because of the continually changing *protein concentration* and accumulation of protein on the membrane surface. The consequent errors, fully discussed by Grollman (110), are in the opposite direction to those postulated by Bennhold. Despite its limitations, ultrafiltration is a fairly accurate indicator of the extent of interaction. For convenient and rapid approximation of the fraction of drug bound in a serum sample it is invaluable.

d *Conductivity and E M F measurements*. These methods are obviously only applicable to the study of electrolytes. The E M F method lends itself beautifully to quantitating the interaction of an ion that can be incorporated into the electrode system (*e g*, chloride) but is otherwise inapplicable. The studies of Pauli and Schön (187) and of Hayasida (117) on chloride binding by serum



proteins illustrate these methods. The determinations must be preceded by removal, so far as possible, of all extraneous ions, so that the use of buffers is consequently much limited.

e *Osmotic pressure, vapor pressure, surface tension, freezing point.* Although they have found little use, these methods are mentioned because they offer theoretically valid possibilities for detecting a reduction in the expected thermodynamic activity of a drug consequent to interaction. The method of osmotic pressure was employed by Scatchard and his associates (220) for studying the interaction of chloride with serum albumin. Lowering of vapor pressure was the basis of some early experiments on the binding of chloroform in serum (173).

Lecomte du Noüy (148, 149) observed that bile salts lowered the surface tension of serum only briefly, the original value being restored over a period of several minutes. If the facts are to be interpreted, as he suggests, in terms of bile salt-protein interaction, one must also accept the unusual slowness of the combination. Employing the same methods, Laporta (146) could not repeat these observations. Tayeau and Rolland (255) made the curious discovery that after extraction of lipids, serum is able to antagonize the surface tension lowering action of ten to fifty times as much bile salt as previously.

Except for the observation of Moore and Roaf (174) that the freezing point of serum was not depressed to the calculated degree by chloroform, no studies by this method have been reported.

f *Differential adsorption.* A substance interacting with plasma protein may fail to be adsorbed in the expected manner to some solid adsorbant like charcoal. Ehrstrom (81) used this fact to characterize the "adsorption power" of the plasma in health and disease. He noted, for example, that while congo red was removed by a given amount of charcoal to an extent not exceeding 8% in normal plasma, the plasma of nephrotics and of cachectic patients with neoplastic disease readily gave up 50% or more of the dye.

The method has not been employed extensively nor is it more than a qualitative indication of the firmness of binding. However, it appears to be, in certain respects at least, a model of what occurs when a reversibly bound substance is fixed by various tissue elements in the body. This analogy was demonstrated by von Jancso (265), and earlier by Schulten (227), who were able to show that the staining of various perfused animal tissues by certain dyes was prevented by the addition of serum to the perfusate.

The process must evidently be viewed as a competition for unbound molecules whose distribution between plasma protein and fixed tissue protein (or other adsorbant) depends upon their relative affinity for the two. With the recent advent of specialized exchange resins and refined techniques of chromatographic adsorption, the method of interaction analysis by differential adsorption may prove very useful.

## 2 *Methods based upon alteration of drug properties*

a *Solubility.* One of the simplest ways to demonstrate a drug-protein interaction is to show that a sparingly-soluble drug dissolves more readily in the presence of protein. Drug is removed from the aqueous phase through combi-

nation so that at equilibrium, when the true solubility in the water has been reached, a considerably greater amount of total drug has *apparently* been dissolved

As early as 1904, Moore and Roaf (173, 174) demonstrated that chloroform, ether and other volatile compounds were more soluble in serum than in saline solution, a fact they attributed to interaction with plasma proteins. A similar difference in solubility is to be observed with ethylene and cyclopropane (183) (cf, however, p 137). Mercury salts were shown to dissolve much more readily in serum than in water (256). Recently it was demonstrated that certain substituted naphthoquinones become more soluble in the presence of various plasma protein fractions. In this case interaction was also demonstrated by a biological technique and results by the two methods were in general agreement (124).

It is now obvious that all the naturally occurring lipid-soluble components of the plasma (cholesterol, fatty acids, steroid hormones, carotenoid pigments, water-insoluble vitamins) are in solution only by virtue of their close association with certain of the plasma globulins (49).

Large solubilizing effects are only to be noted with water-insoluble substances. As most drugs do not fall into this category the method of solubility has not often been applied to the study of drug interactions.

b *Diffusion*. Bennhold (11), refining the early work of Busck, employed a diffusion technique in the study of dye interactions. If an aqueous solution of a monodisperse dye is layered over gelatin the dye will diffuse at a predictable rate over a period of several days. The slower diffusion of albumin can also be determined. It is found that many dyes in albumin solution do not diffuse at the expected rate but travel entirely with the albumin. Paradoxically, a colloidal dye which fails to diffuse into the gel from aqueous solution also travels with albumin. This result is evidently to be attributed to a dual effect of the protein—dispersion of the colloidal particles on the one hand, and interaction with the dispersed dye on the other. Unfortunately Bennhold did not vary dye concentrations through a wide range to determine the upper concentration limit of the apparently complete binding. One may not conclude from these experiments that the dyes were irreversibly bound. In principle the diffusion method is not very different from ultrafiltration, the gel performing the function of a semipermeable membrane which separates rapidly-diffusing small molecules from the colloidal proteins.

c *Stabilization*. Molecules which tend to undergo spontaneous breakdown in aqueous solution may be protected in the presence of plasma protein. Such an effect, resulting from interaction, has been demonstrated in the case of bilirubin (3). It must be proved in every case that no specific stabilizing agent is present in the protein solution.

d *Electrophoresis*. The refinements of the Tiselius apparatus, allowing accurate identification of a number of protein moieties in plasma, with provision for sampling as desired, affords the possibility of determining with which migrating protein component, if any, a given drug is associated, and within what concentration range each association occurs. Precise quantitative relations be-

tween drug concentration and molar binding ratio are not readily obtained Bennhold employed this method as his principal tool in the study of a great many interactions When the substance in question is colored or fluorescent its migration with one or another plasma component is particularly easy to follow This is illustrated by the studies of Schubert (226) on riboflavin, and of Martin (164, 165) on bilirubin

Limiting interpretations to the particular conditions of the experiment and extending observations, when possible, over a concentration range are as important in electrophoresis as in diffusion studies At low concentrations an interaction may appear to involve a single protein exclusively and no free drug may be detected But at higher concentrations other proteins may participate or unbound drug may appear, migrating independently of any protein peak Such behavior was observed by Rawson (201) in the case of Evans blue which combined with albumin in a ratio of 8 moles dye to 1 mole albumin, and then began to associate with an  $\alpha$ -globulin Schubert (226) found that riboflavin, which combined initially with a euglobulin, also interacted with a pseudoglobulin at higher concentration

*e Spectrophotometry* Some colored compounds undergo pronounced changes in their absorption spectra upon combination with protein This effect has long been recognized in the case of various heme pigments acting as enzyme prosthetic groups The so-called "protein error" in the use of certain indicator dyes, first discussed by Sørensen (240), is caused by an alteration in the usual color or intensity as a result of dye-protein combination The absorption peak is shifted (usually toward the red) or the magnitude of the peak absorption is altered (usually diminished), or both Robinson and Hogden (208) showed that serum produces both these changes in the spectrum of phenol red but their observations were largely confined to a narrow pH range near the pK of the dye (7.7) They observed that the protein effect is abolished in alkaline solution and this has been confirmed in the course of investigations by the author (103) The interpretation of such influences of pH upon binding will be discussed at length elsewhere (p 145).

Klotz (136, 139, 140) made the fullest use of this method in studying the binding of azosulfonamides and other anionic dyes Because spectral shifts were large he was able to quantitate the data to obtain estimates of bound and unbound dye in a single protein solution

However, the protein interactions of a number of dyes cause no spectral change An interesting hypothesis intended to explain these differences in dye behavior was advanced by Hartley (116) but numerous unexplained cases remain Methylene blue was shown by the author (103) (dialysis method) to enter into combination with albumin and other protein fractions but to display no spectral change in the visible region However, the substance displays two peaks in the ultraviolet, one of which (at 246 m $\mu$ ) undergoes a sharp increase in magnitude with rising protein concentration Michaelis and Granick (172) observed similar differences between the behavior of visible and ultraviolet spectra of this and other dyes and proposed an explanation of the phenomenon Alterations of the

nation so that at equilibrium, when the true solubility in the water has been reached, a considerably greater amount of total drug has *apparently* been dissolved

As early as 1904, Moore and Roaf (173, 174) demonstrated that chloroform, ether and other volatile compounds were more soluble in serum than in saline solution, a fact they attributed to interaction with plasma proteins. A similar difference in solubility is to be observed with ethylene and cyclopropane (183) (cf., however, p 137). Mercury salts were shown to dissolve much more readily in serum than in water (256). Recently it was demonstrated that certain substituted naphthoquinones become more soluble in the presence of various plasma protein fractions. In this case interaction was also demonstrated by a biological technique and results by the two methods were in general agreement (124).

It is now obvious that all the naturally occurring lipid-soluble components of the plasma (cholesterol, fatty acids, steroid hormones, carotenoid pigments, water-insoluble vitamins) are in solution only by virtue of their close association with certain of the plasma globulins (49).

Large solubilizing effects are only to be noted with water-insoluble substances. As most drugs do not fall into this category the method of solubility has not often been applied to the study of drug interactions.

b *Diffusion* Bennhold (11), refining the early work of Busck, employed a diffusion technique in the study of dye interactions. If an aqueous solution of a monodisperse dye is layered over gelatin the dye will diffuse at a predictable rate over a period of several days. The slower diffusion of albumin can also be determined. It is found that many dyes in albumin solution do not diffuse at the expected rate but travel entirely with the albumin. Paradoxically, a colloidal dye which fails to diffuse into the gel from aqueous solution also travels with albumin. This result is evidently to be attributed to a dual effect of the protein—dispersion of the colloidal particles on the one hand, and interaction with the dispersed dye on the other. Unfortunately Bennhold did not vary dye concentrations through a wide range to determine the upper concentration limit of the apparently complete binding. One may not conclude from these experiments that the dyes were irreversibly bound. In principle the diffusion method is not very different from ultrafiltration, the gel performing the function of a semipermeable membrane which separates rapidly-diffusing small molecules from the colloidal proteins.

c *Stabilization* Molecules which tend to undergo spontaneous breakdown in aqueous solution may be protected in the presence of plasma protein. Such an effect, resulting from interaction, has been demonstrated in the case of bilirubin (3). It must be proved in every case that no specific stabilizing agent is present in the protein solution.

d *Electrophoresis* The refinements of the Tiselius apparatus, allowing accurate identification of a number of protein moieties in plasma, with provision for sampling as desired, affords the possibility of determining with which migrating protein component, if any, a given drug is associated, and within what concentration range each association occurs. Precise quantitative relations be-

It may be concluded that recovery of a drug from a protein precipitate suggests, but does not establish, its firm combination with native protein. Failure to discover a drug in a protein precipitate is not evidence against interaction.

b *Viscosity, electrophoretic mobility, sedimentation rate, and other properties.* Certain interactions which ultimately lead to coagulation may first produce more delicate alterations in protein properties. Increasing viscosity appears to be an early stage in the denaturation process. The interaction of urea with serum albumin has been studied (22, 24, 70, 194) by following such a viscosity change, which occurs well before any turbidity can be observed. The process evidently takes place in two steps, only the first of which is readily reversible by a number of stabilizing agents including fatty acid anions, and sulfate at high concentration.

Anionic detergents have been found to interact with albumin, producing complexes with distinctive mobility in an electric field (156, 194-196), and similarly produced complexes were observed to sediment at an altered rate in the ultracentrifuge (196). The interaction complex containing one atom of mercury and two molecules of albumin, reported by Hughes (131), sediments more rapidly than normal albumin. The formation of this complex has been followed by changes in light scattering and the kinetics of the reaction thereby analyzed (154).

Although limited to a special group of proteins, the measurement of enzyme activity or inhibition provides an extraordinarily sensitive method for the study of interaction. Furthermore, changes produced by denaturants are usually detectable by loss of enzyme activity long before the appearance of altered physical properties.

c *Stabilization of protein.* The capacity of a molecular species to prevent denaturation can be accepted as a criterion of interaction provided direct combination with the denaturant can be excluded. The method has been profitably explored in several investigations from the laboratory of J. M. Luck (2, 22-25, 70, 259). Fatty acids of varying chain length were employed as stabilizing agents for plasma albumin. The progress of thermal as well as urea or guanidine denaturation was followed viscosimetrically, nephelometrically, and by ascertaining the temperature at which gross coagulation occurred in a fixed time. The stabilizing effects of the interacting compounds were so marked as to lead to important new concepts bearing on the molecular requirements for interaction with plasma albumin. Information of considerable practical value in the preservation of human albumin for clinical use also emerged from these studies.

Another interesting example of stabilization through interaction is provided from the field of enzymology, in the work of Koelle (144) who showed that human serum cholinesterase is protected against the denaturant action of di-isopropyl fluorophosphate by physostigmine, which is itself a reversible inhibitor of the enzyme.

## EXPRESSION AND INTERPRETATION OF QUANTITATIVE INTERACTION DATA

### 1 *Binding capacity of the protein and tightness of the binding*

Considerable interest has centered in the finding that many drug-protein interactions can be shown to follow an adsorption isotherm. But much con-

ultraviolet spectra might prove very useful in interaction studies were it not for the limitation imposed on the concentration of protein, whose own absorption in this region soon interferes with the dye spectrum

### 3 *Methods based upon alteration of protein properties or behavior*

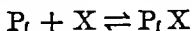
a *Precipitation* The titration of proteins by dyes has been used to determine the available reactive groups on the protein molecule, in confirmation or extension of the results of acid and base titration (90, 199, 200). Experiments with synthetic detergents have yielded very similar results (41, 194, 196). The method generally entails addition of the reagent to complete precipitation of the protein, or back-titration of excess reagent after precipitation. In either case the use of the method is limited to a particular group of apparently stoichiometric, irreversible interactions that ultimately denature the protein. Useful information may be obtained, particularly with regard to the molar combining ratio of the two components. Hewitt (123) carried out such studies with serum albumin and the dyes eosin and rose bengal, finding ratios of 20 and 34 (moles dye per mole albumin), respectively. The principal difficulty in interpreting such data is that the combining ratios thus obtained do not necessarily represent the maximal binding capacity of the native protein. In all likelihood they only indicate the amount of dye required to neutralize the protein net charge to the point of insolubility.

Bound drug can sometimes be determined simply and directly in a protein precipitate. Protein-bound iodine (4, 204, 205, 217), bilirubin in combination with an  $\alpha$ -globulin (49), and the lipids and carbohydrates of plasma lipo- and glyco- proteins (40, 49, 171) appear in precipitates of the respective protein fractions. One investigator (271, 272) has claimed that thrombin-active euglobulin fractions contain firmly bound calcium but this is not generally accepted. Among drugs, suramin (Bayer 205, Germanin) (20, 66, 168, 241), arsphenamine (179), and bismuthyl tartrates (5) have been recovered from protein precipitates.

The objection has been raised that the substance in question may not have been in combination with the native protein before precipitation (59). It is true that more elegant means of demonstrating interaction are often to be preferred but the evidence is clear, at least for naturally-occurring substances, that those found in fractionated protein precipitates are also firmly combined *in vivo*. The tightness of the binding should be stressed here, for substances in easily reversible combination will not be precipitated. On the contrary, coagulating a protein usually abolishes its binding capacity (11, 14, 15, 59, 85, 86, 113, 127), and bound drug is released. This is why most routine chemical methods for determining plasma drug levels give estimates of total (bound and unbound) drug.

An interesting illustration of these generalizations is to be found in the recent studies of Halpern, Dolkart and coworkers (115). Plasma containing benzylpenicillin (G) was fractionated by the method of Cohn *et al* (48) and the fractions assayed for penicillin. The drug was almost quantitatively recovered in the final supernatant despite its well-known binding to albumin *in vivo*. Dissociation of reversible complexes therefore occurs during the preparation and washing of precipitates, when the latter are undenatured.

If it be assumed that all groups on a protein molecule capable of interacting have identical affinities for the drug molecules and that the affinity of any group is unaffected by the combination of drug molecules with other groups, several related equations can be derived from the mass law (137)



$$\frac{(P_i)(X)}{(P_i X)} = K$$

where  $(P_i)$  is the total concentration of *free receptors* (regardless of how many are carried by each protein molecule),  $(X)$  is the concentration of unbound drug,  $(P_i X)$  is the concentration of *combined* receptors, and  $K$  is the dissociation constant. Now let  $n$  be the number of receptor groups carried by each protein molecule, and  $P$  be the molar concentration of total protein. Then  $nP$  represents the total concentration of receptors,  $(P_i X) + (P_i)$ . Substituting  $(P_i) = nP - (P_i X)$ , we find

$$nP(X) - (P_i X)(X) = K(P_i X)$$

$$[K + (X)](P_i X) = nP(X)$$

Now let  $r$  be the moles drug bound per mole total protein, or  $(P_i X)/P$ . Then

$$r = \frac{(P_i X)}{P} = \frac{n(X)}{K + (X)} \quad (1)$$

This is the expression that is identical in form with the Langmuir isotherm.

One important type of interaction experiment is designed to reveal the number of interacting groups on the protein, and the tightness of the binding,  $\epsilon$ ,  $n$  and  $K$  in Equation (1). Such experiments are based upon varying drug concentration and measuring the amounts bound at each concentration by one of the methods previously outlined. To illustrate clearly the results that can be expected when actual data are plotted in various ways, and the interpretation of such data, a hypothetical case will be analyzed. Suppose an interaction experiment in 0.69% albumin solution ( $1 \times 10^{-4}$  M/L), in which 10 binding centers per mole albumin are available to the drug under study. Let the dissociation constant of the drug-albumin complex be  $1 \times 10^{-3}$ . Assume we are able to vary the unbound drug concentration a thousand-fold.

Figure 1 shows the variation in  $r$  as a function of unbound drug concentration, a typical "adsorption-type" curve, asymptotic to 10, the maximal value of  $r$ . The points are chosen at drug concentrations 0.01, 0.1, 1.0 and  $10.0 \times 10^{-3}$  molar and the same symbols are used in this and subsequent figures so that points for high and low drug concentration can be readily identified.

In Figure 2 the same data are plotted according to the method commonly employed for showing conformity to the Freundlich isotherm. The expected linearity is obtained only at low concentrations, when the binding capacity of the protein is but little saturated. The limit approached by the slope ( $\lim_{i \rightarrow 0}$ ) at very low concentration is *always* unity, but with increasing concentration it falls off. Furthermore, the curve over *any* small concentration range would be very nearly linear. These matters are well illustrated in the studies of Grollman (109) on the binding of phenol red by various sera. Varying concentration no more than twenty-fold, this investigator obtained linear log-log plots of different slopes with sera of various species. Obviously his data for each serum fall on a different portion

fusion and not a few irrelevant conclusions have been based upon this simple fact

As a result of his studies with charcoal Freundlich (91) formulated the relationship

$$\frac{x}{m} = kc^{1/n}$$

This is known as the Freundlich isotherm. It states that at a given temperature the amount of a substance adsorbed ( $x$ ) per weight of adsorbant ( $m$ ) is proportional to some power ( $1/n$ ) of the concentration of unadsorbed material ( $c$ ). A number of investigators have expressed their interaction data in terms of this equation, usually plotting the logarithm of  $x/m$  against the logarithm of  $c$  to obtain a straight line of slope  $1/n$ , whose position on the axes gives  $k$ .

It is important to note that the Freundlich isotherm is a purely empirical function. It has no thermodynamic validity and the constants are without special physical significance. The simplest proof of its incorrectness is the fact that it has no end-point. It implies infinite adsorption with increasing concentration of solute, an absurdity since the finite adsorbant surface must ultimately become saturated.

The Langmuir isotherm (145, 145a), on the other hand, is a theoretically valid expression of the facts of adsorption. The equation is

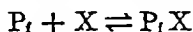
$$y = \frac{x}{m} = \frac{bc}{\frac{1}{a} + c}$$

where  $x/m$  and  $c$  have the same significance as above, while  $a$  and  $b$  are constants whose values can be rationally interpreted. The equation correctly expresses the fact that, at very high values of  $c$ , the adsorbant becomes saturated, the whole term on the right approaching a limiting value  $b$ . The Langmuir isotherm is entirely equivalent to the mass law equilibrium (cf p 113), the more usual form for characterizing the reversible association of two components. A more involved treatment of multi-layer adsorption was proposed by Brunauer, Emmett and Teller (29), and has been applied to the binding of water vapor by dry plasma (and other) proteins (32).

It seems appropriate to restate an important point about adsorption previously emphasized with considerable passion in an entertaining review by Mathews (166) about twenty-five years ago. If a substance is concentrated reversibly at a surface we say it is adsorbed. The term is simply descriptive of this fact and does not imply anything about the nature of the responsible forces. Therefore the mere finding that an adsorption isotherm is followed *does not mean* that the forces are physical rather than chemical in nature. "There is no present justification for dividing interatomic (or intermolecular) forces into *physical* and *chemical* forces. It is much more profitable to consider all such forces as strictly chemical in nature." This statement, made over thirty years ago by Langmuir (145), has its counterpart in the identity of his adsorption isotherm with the law of mass action.



If it be assumed that all groups on a protein molecule capable of interacting have identical affinities for the drug molecules and that the affinity of any group is unaffected by the combination of drug molecules with other groups, several related equations can be derived from the mass law (137)



$$\frac{(P_i)(X)}{(P_i X)} = K$$

where  $(P_i)$  is the total concentration of *free receptors* (regardless of how many are carried by each protein molecule),  $(X)$  is the concentration of unbound drug,  $(P_i X)$  is the concentration of *combined* receptors, and  $K$  is the dissociation constant. Now let  $n$  be the number of receptor groups carried by each protein molecule, and  $P$  be the molar concentration of total protein. Then  $nP$  represents the total concentration of receptors,  $(P_i X) + (P_i)$ . Substituting  $(P_i) = nP - (P_i X)$ , we find

$$nP(X) - (P_i X)(X) = K(P_i X)$$

$$[K + (X)](P_i X) = nP(X)$$

Now let  $r$  be the moles drug bound per mole total protein, or  $(P_i X)/P$ . Then

$$r = \frac{(P_i X)}{P} = \frac{n(X)}{K + (X)} \quad (1)$$

This is the expression that is identical in form with the Langmuir isotherm

One important type of interaction experiment is designed to reveal the number of interacting groups on the protein, and the tightness of the binding, *i.e.*,  $n$  and  $K$  in Equation (1). Such experiments are based upon varying drug concentration and measuring the amounts bound at each concentration by one of the methods previously outlined. To illustrate clearly the results that can be expected when actual data are plotted in various ways, and the interpretation of such data, a hypothetical case will be analyzed. Suppose an interaction experiment in 0.69% albumin solution ( $1 \times 10^{-4}$  M/L), in which 10 binding centers per mole albumin are available to the drug under study. Let the dissociation constant of the drug-albumin complex be  $1 \times 10^{-3}$ . Assume we are able to vary the unbound drug concentration a thousand-fold.

Figure 1 shows the variation in  $r$  as a function of unbound drug concentration, a typical "adsorption-type" curve, asymptotic to 10, the maximal value of  $r$ . The points are chosen at drug concentrations 0.01, 0.1, 1.0 and  $10.0 \times 10^{-3}$  molar and the same symbols are used in this and subsequent figures so that points for high and low drug concentration can be readily identified.

In Figure 2 the same data are plotted according to the method commonly employed for showing conformity to the Freundlich isotherm. The expected linearity is obtained only at low concentrations, when the binding capacity of the protein is but little saturated. The limit approached by the slope ( $\lim_{1/n}$ ) at very low concentration is *always* unity, but with increasing concentration it falls off. Furthermore, the curve over *any* small concentration range would be very nearly linear. These matters are well illustrated in the studies of Grollman (109) on the binding of phenol red by various sera. Varying concentration no more than twenty-fold, this investigator obtained linear log-log plots of different slopes with sera of various species. Obviously his data for each serum fall on a different portion

fusion and not a few irrelevant conclusions have been based upon this simple fact

As a result of his studies with charcoal Freundlich (91) formulated the relationship

$$\frac{x}{m} = kc^{1/n}$$

This is known as the Freundlich isotherm. It states that at a given temperature the amount of a substance adsorbed ( $x$ ) per weight of adsorbant ( $m$ ) is proportional to some power ( $1/n$ ) of the concentration of unadsorbed material ( $c$ ). A number of investigators have expressed their interaction data in terms of this equation, usually plotting the logarithm of  $x/m$  against the logarithm of  $c$  to obtain a straight line of slope  $1/n$ , whose position on the axes gives  $k$ .

It is important to note that the Freundlich isotherm is a purely empirical function. It has no thermodynamic validity and the constants are without special physical significance. The simplest proof of its incorrectness is the fact that it has no end-point. It implies infinite adsorption with increasing concentration of solute, an absurdity since the finite adsorbant surface must ultimately become saturated.

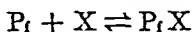
The Langmuir isotherm (145, 145a), on the other hand, is a theoretically valid expression of the facts of adsorption. The equation is

$$y = \frac{x}{m} = \frac{bc}{\frac{1}{a} + c}$$

where  $x/m$  and  $c$  have the same significance as above, while  $a$  and  $b$  are constants whose values can be rationally interpreted. The equation correctly expresses the fact that, at very high values of  $c$ , the adsorbant becomes saturated, the whole term on the right approaching a limiting value  $b$ . The Langmuir isotherm is entirely equivalent to the mass law equilibrium (cf p 113), the more usual form for characterizing the reversible association of two components. A more involved treatment of multi-layer adsorption was proposed by Brunauer, Emmett and Teller (29), and has been applied to the binding of water vapor by dry plasma (and other) proteins (32).

It seems appropriate to restate an important point about adsorption previously emphasized with considerable passion in an entertaining review by Mathews (166) about twenty-five years ago. If a substance is concentrated reversibly at a surface we say it is adsorbed. The term is simply descriptive of this fact and does not imply anything about the nature of the responsible forces. Therefore the mere finding that an adsorption isotherm is followed *does not mean* that the forces are physical rather than chemical in nature. "There is no present justification for dividing interatomic (or intermolecular) forces into *physical* and *chemical* forces. It is much more profitable to consider all such forces as strictly chemical in nature." This statement, made over thirty years ago by Langmuir (145), has its counterpart in the identity of his adsorption isotherm with the law of mass action.

If it be assumed that all groups on a protein molecule capable of interacting have identical affinities for the drug molecules and that the affinity of any group is unaffected by the combination of drug molecules with other groups, several related equations can be derived from the mass law (137)



$$\frac{(P_i)(X)}{(P_i X)} = K$$

where  $(P_i)$  is the total concentration of *free receptors* (regardless of how many are carried by each protein molecule),  $(X)$  is the concentration of unbound drug,  $(P_i X)$  is the concentration of *combined* receptors, and  $K$  is the dissociation constant. Now let  $n$  be the number of receptor groups carried by each protein molecule, and  $P$  be the molar concentration of total protein. Then  $nP$  represents the total concentration of receptors,  $(P_i X) + (P_i)$ . Substituting  $(P_i) = nP - (P_i X)$ , we find

$$nP(X) - (P_i X)(X) = K(P_i X)$$

$$[K + (X)](P_i X) = nP(X)$$

Now let  $r$  be the moles drug bound per mole total protein, or  $(P_i X)/P$ . Then

$$r = \frac{(P_i X)}{P} = \frac{n(X)}{K + (X)} \quad (1)$$

This is the expression that is identical in form with the Langmuir isotherm

One important type of interaction experiment is designed to reveal the number of interacting groups on the protein, and the tightness of the binding,  $\epsilon$ ,  $n$  and  $K$  in Equation (1). Such experiments are based upon varying drug concentration and measuring the amounts bound at each concentration by one of the methods previously outlined. To illustrate clearly the results that can be expected when actual data are plotted in various ways, and the interpretation of such data, a hypothetical case will be analyzed. Suppose an interaction experiment in 0.69% albumin solution ( $1 \times 10^{-4}$  M/L), in which 10 binding centers per mole albumin are available to the drug under study. Let the dissociation constant of the drug-albumin complex be  $1 \times 10^{-3}$ . Assume we are able to vary the unbound drug concentration a thousand-fold.

Figure 1 shows the variation in  $r$  as a function of unbound drug concentration, a typical "adsorption-type" curve, asymptotic to 10, the maximal value of  $r$ . The points are chosen at drug concentrations 0.01, 0.1, 1.0 and  $10.0 \times 10^{-3}$  molar and the same symbols are used in this and subsequent figures so that points for high and low drug concentration can be readily identified.

In Figure 2 the same data are plotted according to the method commonly employed for showing conformity to the Freundlich isotherm. The expected linearity is obtained only at low concentrations, when the binding capacity of the protein is but little saturated. The limit approached by the slope  $(\lim_{1/n})$  at very low concentration is *always* unity, but with increasing concentration it falls off. Furthermore, the curve over any small concentration range would be very nearly linear. These matters are well illustrated in the studies of Grollman (109) on the binding of phenol red by various sera. Varying concentration no more than twenty-fold, this investigator obtained linear log-log plots of different slopes with sera of various species. Obviously his data for each serum fall on a different portion

fusion and not a few irrelevant conclusions have been based upon this simple fact

As a result of his studies with charcoal Freundlich (91) formulated the relationship

$$\frac{x}{m} = kc^{1/n}$$

This is known as the Freundlich isotherm. It states that at a given temperature the amount of a substance adsorbed ( $x$ ) per weight of adsorbant ( $m$ ) is proportional to some power ( $1/n$ ) of the concentration of unadsorbed material ( $c$ ). A number of investigators have expressed their interaction data in terms of this equation, usually plotting the logarithm of  $x/m$  against the logarithm of  $c$  to obtain a straight line of slope  $1/n$ , whose position on the axes gives  $k$ .

It is important to note that the Freundlich isotherm is a purely empirical function. It has no thermodynamic validity and the constants are without special physical significance. The simplest proof of its incorrectness is the fact that it has no end-point. It implies infinite adsorption with increasing concentration of solute, an absurdity since the finite adsorbant surface must ultimately become saturated.

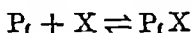
The Langmuir isotherm (145, 145a), on the other hand, is a theoretically valid expression of the facts of adsorption. The equation is

$$y = \frac{x}{m} = \frac{bc}{\frac{1}{a} + c}$$

where  $x/m$  and  $c$  have the same significance as above, while  $a$  and  $b$  are constants whose values can be rationally interpreted. The equation correctly expresses the fact that, at very high values of  $c$ , the adsorbant becomes saturated, the whole term on the right approaching a limiting value  $b$ . The Langmuir isotherm is entirely equivalent to the mass law equilibrium (cf p 113), the more usual form for characterizing the reversible association of two components. A more involved treatment of multi-layer adsorption was proposed by Brunauer, Emmett and Teller (29), and has been applied to the binding of water vapor by dry plasma (and other) proteins (32).

It seems appropriate to restate an important point about adsorption previously emphasized with considerable passion in an entertaining review by Mathews (166) about twenty-five years ago. If a substance is concentrated reversibly at a surface we say it is adsorbed. The term is simply descriptive of this fact and does not imply anything about the nature of the responsible forces. Therefore the mere finding that an adsorption isotherm is followed *does not mean* that the forces are physical rather than chemical in nature. "There is no present justification for dividing interatomic (or intermolecular) forces into *physical* and *chemical* forces. It is much more profitable to consider all such forces as strictly chemical in nature." This statement, made over thirty years ago by Langmuir (145), has its counterpart in the identity of his adsorption isotherm with the law of mass action.

If it be assumed that all groups on a protein molecule capable of interacting have identical affinities for the drug molecules and that the affinity of any group is unaffected by the combination of drug molecules with other groups, several related equations can be derived from the mass law (137)



$$\frac{(P_f)(X)}{(P_f X)} = K$$

where  $(P_f)$  is the total concentration of *free receptors* (regardless of how many are carried by each protein molecule),  $(X)$  is the concentration of unbound drug,  $(P_f X)$  is the concentration of *combined* receptors, and  $K$  is the dissociation constant. Now let  $n$  be the number of receptor groups carried by each protein molecule, and  $P$  be the molar concentration of total protein. Then  $nP$  represents the total concentration of receptors,  $(P_f X) + (P_f)$ . Substituting  $(P_f) = nP - (P_f X)$ , we find

$$nP(X) - (P_f X)(X) = K(P_f X)$$

$$[K + (X)](P_f X) = nP(X)$$

Now let  $r$  be the moles drug bound per mole total protein, or  $(P_f X)/P$ . Then

$$r = \frac{(P_f X)}{P} = \frac{n(X)}{K + (X)} \quad (1)$$

This is the expression that is identical in form with the Langmuir isotherm.

One important type of interaction experiment is designed to reveal the number of interacting groups on the protein, and the tightness of the binding,  $r$ ,  $n$  and  $K$  in Equation (1). Such experiments are based upon varying drug concentration and measuring the amounts bound at each concentration by one of the methods previously outlined. To illustrate clearly the results that can be expected when actual data are plotted in various ways, and the interpretation of such data, a hypothetical case will be analyzed. Suppose an interaction experiment in 0.69% albumin solution ( $1 \times 10^{-4}$  M/L), in which 10 binding centers per mole albumin are available to the drug under study. Let the dissociation constant of the drug-albumin complex be  $1 \times 10^{-3}$ . Assume we are able to vary the unbound drug concentration a thousand-fold.

Figure 1 shows the variation in  $r$  as a function of unbound drug concentration, a typical "adsorption type" curve, asymptotic to 10, the maximal value of  $r$ . The points are chosen at drug concentrations 0.01, 0.1, 1.0 and  $10.0 \times 10^{-3}$  molar and the same symbols are used in this and subsequent figures so that points for high and low drug concentration can be readily identified.

In Figure 2 the same data are plotted according to the method commonly employed for showing conformity to the Freundlich isotherm. The expected linearity is obtained only at low concentrations, when the binding capacity of the protein is but little saturated. The limit approached by the slope ( $\log_{10} r$ ) at very low concentration is *always* unity, but with increasing concentration it falls off. Furthermore, the curve over *any* small concentration range would be very nearly linear. These matters are well illustrated in the studies of Grollman (109) on the binding of phenol red by various sera. Varying concentration no more than twenty-fold, this investigator obtained linear log-log plots of different slopes with sera of various species. Obviously his data for each serum fall on a different portion

fusion and not a few irrelevant conclusions have been based upon this simple fact

As a result of his studies with charcoal Freundlich (91) formulated the relationship

$$\frac{x}{m} = kc^{1/n}$$

This is known as the Freundlich isotherm. It states that at a given temperature the amount of a substance adsorbed ( $x$ ) per weight of adsorbant ( $m$ ) is proportional to some power ( $1/n$ ) of the concentration of unadsorbed material ( $c$ ). A number of investigators have expressed their interaction data in terms of this equation, usually plotting the logarithm of  $x/m$  against the logarithm of  $c$  to obtain a straight line of slope  $1/n$ , whose position on the axes gives  $k$ .

It is important to note that the Freundlich isotherm is a purely empirical function. It has no thermodynamic validity and the constants are without special physical significance. The simplest proof of its incorrectness is the fact that it has no end-point. It implies infinite adsorption with increasing concentration of solute, an absurdity since the finite adsorbant surface must ultimately become saturated.

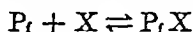
The Langmuir isotherm (145, 145a), on the other hand, is a theoretically valid expression of the facts of adsorption. The equation is

$$y = \frac{x}{m} = \frac{bc}{\frac{1}{a} + c}$$

where  $x/m$  and  $c$  have the same significance as above, while  $a$  and  $b$  are constants whose values can be rationally interpreted. The equation correctly expresses the fact that, at very high values of  $c$ , the adsorbant becomes saturated, the whole term on the right approaching a limiting value  $b$ . The Langmuir isotherm is entirely equivalent to the mass law equilibrium (cf p 113), the more usual form for characterizing the reversible association of two components. A more involved treatment of multi-layer adsorption was proposed by Brunauer, Emmett and Teller (29), and has been applied to the binding of water vapor by dry plasma (and other) proteins (32).

It seems appropriate to restate an important point about adsorption previously emphasized with considerable passion in an entertaining review by Mathews (166) about twenty-five years ago. If a substance is concentrated reversibly at a surface we say it is adsorbed. The term is simply descriptive of this fact and does not imply anything about the nature of the responsible forces. Therefore the mere finding that an adsorption isotherm is followed *does not mean* that the forces are physical rather than chemical in nature. "There is no present justification for dividing interatomic (or intermolecular) forces into *physical* and *chemical* forces. It is much more profitable to consider all such forces as strictly chemical in nature." This statement, made over thirty years ago by Langmuir (145), has its counterpart in the identity of his adsorption isotherm with the law of mass action.

If it be assumed that all groups on a protein molecule capable of interacting have identical affinities for the drug molecules and that the affinity of any group is unaffected by the combination of drug molecules with other groups, several related equations can be derived from the mass law (137)



$$\frac{(P_f)(X)}{(P_f X)} = K$$

where  $(P_f)$  is the total concentration of *free receptors* (regardless of how many are carried by each protein molecule),  $(X)$  is the concentration of unbound drug,  $(P_f X)$  is the concentration of *combined* receptors, and  $K$  is the dissociation constant. Now let  $n$  be the number of receptor groups carried by each protein molecule, and  $P$  be the molar concentration of total protein. Then  $nP$  represents the total concentration of receptors,  $(P_f X) + (P_f)$ . Substituting  $(P_f) = nP - (P_f X)$ , we find

$$nP(X) - (P_f X)(X) = K(P_f X)$$

$$[K + (X)](P_f X) = nP(X)$$

Now let  $r$  be the moles drug bound per mole total protein, or  $(P_f X)/P$ . Then

$$r = \frac{(P_f X)}{P} = \frac{n(X)}{K + (X)} \quad (1)$$

This is the expression that is identical in form with the Langmuir isotherm

One important type of interaction experiment is designed to reveal the number of interacting groups on the protein, and the tightness of the binding,  $\epsilon$ ,  $n$  and  $K$  in Equation (1). Such experiments are based upon varying drug concentration and measuring the amounts bound at each concentration by one of the methods previously outlined. To illustrate clearly the results that can be expected when actual data are plotted in various ways, and the interpretation of such data, a hypothetical case will be analyzed. Suppose an interaction experiment in 0.69% albumin solution ( $1 \times 10^{-4}$  M/L), in which 10 binding centers per mole albumin are available to the drug under study. Let the dissociation constant of the drug-albumin complex be  $1 \times 10^{-3}$ . Assume we are able to vary the unbound drug concentration a thousand-fold.

Figure 1 shows the variation in  $r$  as a function of unbound drug concentration, a typical "adsorption-type" curve, asymptotic to 10, the maximal value of  $r$ . The points are chosen at drug concentrations 0.01, 0.1, 1.0 and  $10.0 \times 10^{-3}$  molar and the same symbols are used in this and subsequent figures so that points for high and low drug concentration can be readily identified.

In Figure 2 the same data are plotted according to the method commonly employed for showing conformity to the Freundlich isotherm. The expected linearity is obtained only at low concentrations, when the binding capacity of the protein is but little saturated. The limit approached by the slope ( $\lim_{\mu \rightarrow 0}$ ) at very low concentration is *always* unity, but with increasing concentration it falls off. Furthermore, the curve over *any* small concentration range would be very nearly linear. These matters are well illustrated in the studies of Grollman (109) on the binding of phenol red by various sera. Varying concentration no more than twenty-fold, this investigator obtained linear log-log plots of different slopes with sera of various species. Obviously his data for each serum fall on a different portion

fusion and not a few irrelevant conclusions have been based upon this simple fact

As a result of his studies with charcoal Freundlich (91) formulated the relationship

$$\frac{x}{m} = kc^{1/n}$$

This is known as the Freundlich isotherm. It states that at a given temperature the amount of a substance adsorbed ( $x$ ) per weight of adsorbant ( $m$ ) is proportional to some power ( $1/n$ ) of the concentration of unadsorbed material ( $c$ ). A number of investigators have expressed their interaction data in terms of this equation, usually plotting the logarithm of  $x/m$  against the logarithm of  $c$  to obtain a straight line of slope  $1/n$ , whose position on the axes gives  $k$ .

It is important to note that the Freundlich isotherm is a purely empirical function. It has no thermodynamic validity and the constants are without special physical significance. The simplest proof of its incorrectness is the fact that it has no end-point. It implies infinite adsorption with increasing concentration of solute, an absurdity since the finite adsorbant surface must ultimately become saturated.

The Langmuir isotherm (145, 145a), on the other hand, is a theoretically valid expression of the facts of adsorption. The equation is

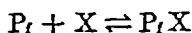
$$y = \frac{x}{m} = \frac{bc}{\frac{1}{a} + c}$$

where  $x/m$  and  $c$  have the same significance as above, while  $a$  and  $b$  are constants whose values can be rationally interpreted. The equation correctly expresses the fact that, at very high values of  $c$ , the adsorbant becomes saturated, the whole term on the right approaching a limiting value  $b$ . The Langmuir isotherm is entirely equivalent to the mass law equilibrium (cf p 113), the more usual form for characterizing the reversible association of two components. A more involved treatment of multi-layer adsorption was proposed by Brunauer, Emmett and Teller (29), and has been applied to the binding of water vapor by dry plasma (and other) proteins (32).

It seems appropriate to restate an important point about adsorption previously emphasized with considerable passion in an entertaining review by Mathews (166) about twenty-five years ago. If a substance is concentrated reversibly at a surface we say it is adsorbed. The term is simply descriptive of this fact and does not imply anything about the nature of the responsible forces. Therefore the mere finding that an adsorption isotherm is followed *does not mean* that the forces are physical rather than chemical in nature. "There is no present justification for dividing interatomic (or intermolecular) forces into *physical* and *chemical* forces. It is much more profitable to consider all such forces as strictly chemical in nature." This statement, made over thirty years ago by Langmuir (145), has its counterpart in the identity of his adsorption isotherm with the law of mass action.



If it be assumed that all groups on a protein molecule capable of interacting have identical affinities for the drug molecules and that the affinity of any group is unaffected by the combination of drug molecules with other groups, several related equations can be derived from the mass law (137)



$$\frac{(P_t)(X)}{(P_t X)} = K$$

where  $(P_t)$  is the total concentration of *free receptors* (regardless of how many are carried by each protein molecule),  $(X)$  is the concentration of unbound drug,  $(P_t X)$  is the concentration of *combined* receptors, and  $K$  is the dissociation constant. Now let  $n$  be the number of receptor groups carried by each protein molecule, and  $P$  be the molar concentration of total protein. Then  $nP$  represents the total concentration of receptors,  $(P_t X) + (P_t)$ . Substituting  $(P_t) = nP - (P_t X)$ , we find

$$nP(X) - (P_t X)(X) = K(P_t X)$$

$$[K + (X)](P_t X) = nP(X)$$

Now let  $r$  be the moles drug bound per mole total protein, or  $(P_t X)/P$ . Then

$$r = \frac{(P_t X)}{P} = \frac{n(X)}{K + (X)} \quad (1)$$

This is the expression that is identical in form with the Langmuir isotherm

One important type of interaction experiment is designed to reveal the number of interacting groups on the protein, and the tightness of the binding,  $r$ ,  $e$ ,  $n$  and  $K$  in Equation (1). Such experiments are based upon varying drug concentration and measuring the amounts bound at each concentration by one of the methods previously outlined. To illustrate clearly the results that can be expected when actual data are plotted in various ways, and the interpretation of such data, a hypothetical case will be analyzed. Suppose an interaction experiment in 0.69% albumin solution ( $1 \times 10^{-4}$  M/L), in which 10 binding centers per mole albumin are available to the drug under study. Let the dissociation constant of the drug-albumin complex be  $1 \times 10^{-3}$ . Assume we are able to vary the unbound drug concentration a thousand-fold.

Figure 1 shows the variation in  $r$  as a function of unbound drug concentration, a typical "adsorption-type" curve, asymptotic to 10, the maximal value of  $r$ . The points are chosen at drug concentrations 0.01, 0.1, 1.0 and  $10.0 \times 10^{-3}$  molar and the same symbols are used in this and subsequent figures so that points for high and low drug concentration can be readily identified.

In Figure 2 the same data are plotted according to the method commonly employed for showing conformity to the Freundlich isotherm. The expected linearity is obtained only at low concentrations, when the binding capacity of the protein is but little saturated. The limit approached by the slope ( $\lim_{\mu \rightarrow 0}$ ) at very low concentration is *always* unity, but with increasing concentration it falls off. Furthermore, the curve over *any* small concentration range would be very nearly linear. These matters are well illustrated in the studies of Grollman (109) on the binding of phenol red by various sera. Varying concentration no more than twenty-fold, this investigator obtained linear log-log plots of different slopes with sera of various species. Obviously his data for each serum fall on a different portion

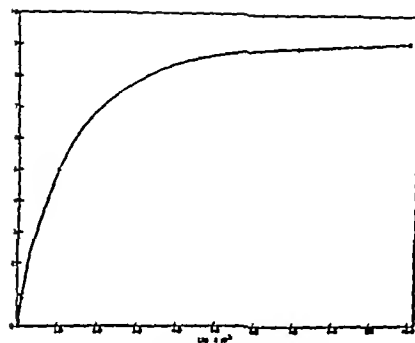


Fig 1

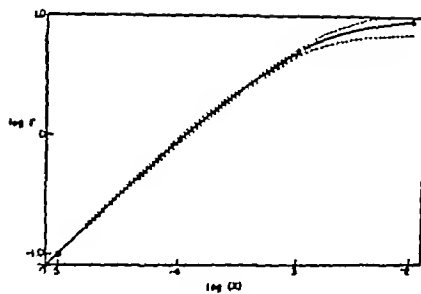


Fig 2

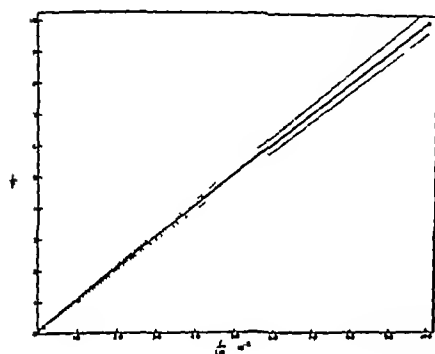


Fig 3

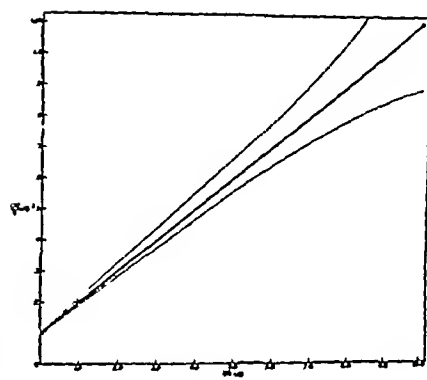


Fig 4

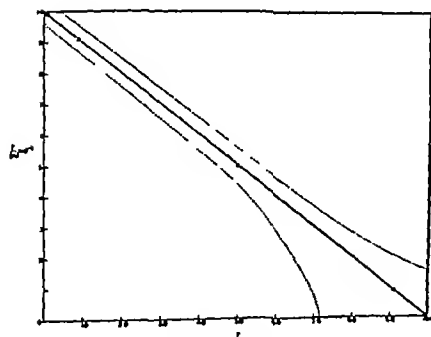


Fig 5

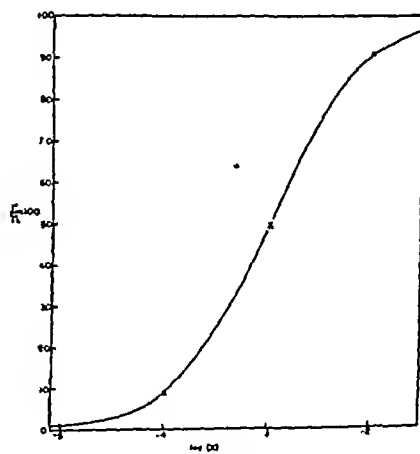


Fig 6

Figs 1-6 For explanation see text

of the true curve and apparent conformity to the Freundlich isotherm can only be attributed to the narrow concentration range he employed

Equation (1) can be rearranged in a number of ways for convenient plotting to yield values of  $K$  and  $n$  by extrapolation. If reciprocals of  $r$  and  $(X)$  are plotted, as in *Figure 3*, one obtains a straight line with slope  $K/n$  and intercept  $1/n$ . This form will be familiar to enzymologists as the Lineweaver-Burk plot (152) and its use in interaction studies is well illustrated in Greenberg's (106) review of cation-protein interactions. In this and the other figures the disproportionate weighting of points representing high and low drug concentration should be noted. Here a few *low* drug concentrations will outweigh many high ones. The broken lines indicate the possible variation resulting from a maximum error of  $\pm 1\%$  in the determination of drug concentration. The differing appearances of this variation on the several plots should be especially observed.

In *Figure 4*,  $(X)/r$  is plotted against  $(X)$ , as in the investigations of Klotz (137, 138). Here the slope is  $1/n$  and the intercept  $K/n$ . In contrast to *Figure 3*, *high* drug concentrations here weigh especially heavily.

*Figure 5* represents the type of plot advocated by Scatchard (221a), in which the ordinal and abscissal intercepts give, respectively,  $n/K$  and  $n$ .

In *Figure 6*,  $100 \times r/n$  is plotted against  $\log (X)$ . When a negligible fraction of the total drug is bound the abscissae can be expressed as "log total drug concentration." This is then the familiar dose-response curve or, alternatively, the course of saturation of an enzyme by its substrate or inhibitor, the ordinates representing per cent saturation of available groups.

Regardless of the manner of rearranging the equation it will not accurately describe a situation which fails to conform to the principal underlying assumptions.

(1) The interaction must be reversible and the data must be obtained at complete equilibrium.

(2) All groups must have the same affinity for the drug. If certain groups react more avidly than others, *sharp* breaks in the various linear curves will be obvious only if the affinities of the several species of receptor differ widely. Although this question has been scantily explored, it seems very likely that affinity of drugs to various binding centers does vary. The data of Thumann and Rothschild (260) on the interaction of indole-acetate with plasma albumin suggest that the first interacting groups on each albumin molecule bind more firmly than subsequent ones. Similar complex behavior might be expected in the case of chloride and thiocyanate, under study at present by Scatchard and coworkers (221, 222). At equivalent concentrations 24 moles of thiocyanate but only 6 moles of chloride are bound to albumin. If, as seems probable, the groups binding chloride are included among those binding thiocyanate, it might follow that thiocyanate binding involves at least two species of receptors of different affinity. This, however, has not yet been demonstrated.

(3) Systematic deviation of the data in the direction of reduced binding may result from electrostatic repulsion, by initially bound drug, of the approach of subsequent ions of the same charge. Such effects, which may occur with multivalent ions (and which can be corrected for), were observed by Klotz (138, 140) in the case of di- and tri-sulfonated azo dyes.

(4) If drug concentration is not varied through a sufficient range the extrapolations will not even approximate the true maximum number of available groups.

but will only show the number of groups readily available, *i.e.*, those with highest affinity for the drug molecules. Such information is valuable but should not be misinterpreted.

(5) The quantitative methods described here are obviously only applicable to interactions with purified and homogeneous proteins of known molecular weight. In view of the widely differing combining power of diverse proteins it is extremely hazardous to attempt an estimate of molar binding capacity in the presence of even the smallest quantities of contaminant protein.

## 2 *The ratio of bound to total drug*

In the foregoing discussion prime attention was focussed upon the protein molecule and the course of its saturation by an interacting drug. There is good reason to examine the problem also from the standpoint of the drug molecules and their partition between bound and unbound forms.<sup>2</sup> This partition largely determines the degree to which pharmacological properties of a drug can be modified by interaction with plasma proteins. A further interest in this approach arises from the frequent inapplicability of the techniques at hand to the kind of saturation experiments already described, either because of impurity of the proteins or because drug concentration cannot be suitably varied.

It is therefore desirable to know how maximal binding capacity and tightness of binding determine the ratio of bound to total drug. Bechhold (7) long ago stated the basic principle that large effects upon this ratio are to be observed, as a rule, only when *few* of the available protein groups have been saturated, *i.e.*, at low drug concentration.

Equation (1) can be rearranged to yield an expression for the fraction  $\frac{\text{bound drug}}{\text{total drug}}$ , which we shall designate by the symbol  $\beta$

$$\begin{aligned} \frac{(X)}{(P_1X)} &= \frac{K + (X)}{nP} \\ \beta &= \frac{(P_1X)}{(P_1X) + (X)} = \frac{1}{1 + \frac{K}{nP} + \frac{(X)}{nP}} \end{aligned} \quad (2)$$

This equation states that the *fraction bound* depends upon the protein concentration, the drug concentration, the dissociation constant of the drug-protein complex, and the number of groups available for drug binding on each protein molecule. Either  $K$  or  $n$ , or both, may be affected by pH, ionic strength and temperature. Consequently the values of these variables should always be reported.

<sup>2</sup> The term "free drug" is employed in two utterly unrelated contexts. It is meant to distinguish a drug in its original state from the same drug after conjugation (*e.g.*, "free sulfonamide" as opposed to conjugated sulfonamide). It is also used to connote unbound as opposed to protein-bound drug. While the latter sense would conform to general usage in physical chemistry, the former is well established in pharmacology. It will probably lead to a minimum of confusion if the term "unbound" be uniformly adopted. This convention will be followed throughout this paper.

Figure 7 is the graphical representation of Equation (2), where  $\beta$  is plotted as a function of  $\log (X)/nP$  for discrete values of  $K/nP$ . For each particular drug-protein system a single one of these curves will apply, the abscissae being then proportional to the logarithms of unbound drug concentrations. From the equation and figure, the following behavior can be predicted

(1) As the protein concentration increases,  $\beta$  also increases toward unity. Regardless of any other factors, all interacting drugs should be *completely bound* at high enough protein concentration, but in real instances, when affinity is low, such concentrations might be out of the question. Conversely, dilution of a system containing bound drug results in dissociation of the complex

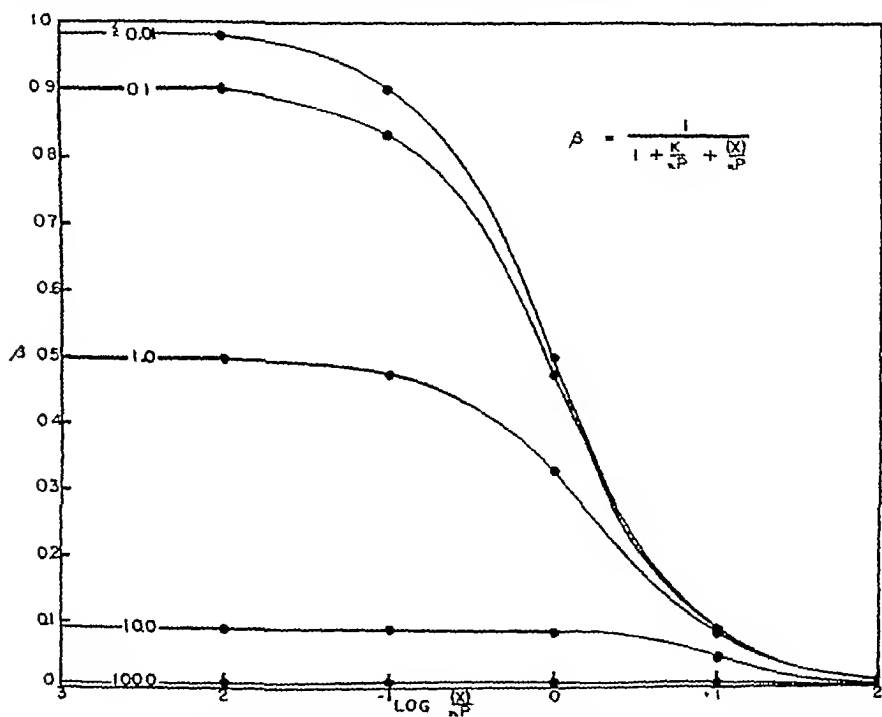


FIG 7 FRACTION BOUND ( $\beta$ ) AS A FUNCTION OF  $\log (X)/nP$  FOR DISCRETE VALUES OF  $K/nP$

(2) Drug concentration is crucial. As it is increased more drug is bound per protein molecule, but at the same time the *fraction bound decreases*. Inspection of the equation and figure shows that if the drug concentration is high enough essentially all the drug is unbound in a system held constant in other respects. Conversely, as drug concentration falls, the fraction bound approaches a maximum. This maximum is not necessarily unity but is represented by the term  $\frac{1}{1 + K/nP}$ . Consequently for certain interactions the maximal fraction bound, at very low drug concentration, may be extremely small while for others (e.g., if  $K/nP < 0.1$ ) the binding may be practically complete. The statement that a

given fraction of drug is bound in the plasma is meaningless unless qualified by an indication of the unbound drug concentration at equilibrium. If conclusions bearing upon pharmacological action are to be drawn, interaction studies must be carried out at therapeutic drug levels, for experiments at arbitrary higher levels will usually (but not necessarily) give underestimates of  $\beta$ .

(3) The smaller the dissociation constant the tighter will be the binding and the greater the fraction bound. On the other hand the equation shows that a small  $K$  can be offset by a large  $(X)$ , so that at high enough concentration essentially all of a drug will be present in unbound form. As noted above, if  $K$  is considerably smaller than  $nP$ , the whole term containing  $K$  becomes negligible. This means that all drugs with very high affinity should behave identically. Affinity differences will be obscured and fractional binding will depend upon the drug concentration alone. All such drugs will be completely bound at comparably low concentrations.

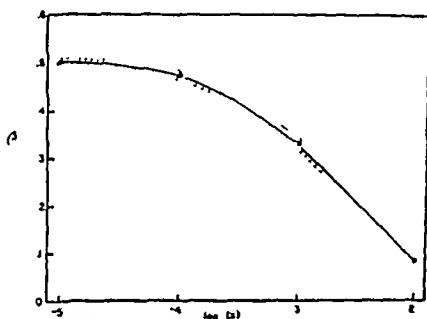


FIG 8 For explanation see text

(4) Other things being equal, the greater the total number of receptor groups per molecule, the larger will be the fractional binding of interacting molecules.

In Figure 8 the hypothetical interaction analyzed in the previous section is plotted in terms of  $\beta$ . The curve, as was to be expected, is identical with that of Figure 7 for which  $K/nP = 1$ , and the maximal binding is 0.5. Frequently it is only possible to investigate an interaction at low drug concentrations. If, in such a case, two measurements at concentrations differing by a factor of ten give the same fractional binding, it can safely be assumed that the maximal value of  $\beta$  has been nearly reached. The system can therefore be characterized in certain important respects, and the ratio  $K/n$  obtained, even though distinct values for each constant cannot be determined. It is to be recognized that every interaction will show an unchanging, maximal value of  $\beta$  below a certain drug concentration. For example, the finding that chloromycetin is 45% bound to crystalline bovine albumin through a considerable range of concentration, reported by Smith and his associates (238) as an anomalous finding, should in fact have been expected, and could have been used to derive information of some quantitative value about the interaction.

Frequently the concentrations of protein and of total drug are varied simultaneously to determine what molar ratio gives a certain constant effect. Davis

and Dubos (56, 58) observed that expected antibacterial action could be prevented at several different concentrations of oleic acid and albumin provided the molar ratio oleic acid/albumin = 3 was not exceeded, while the ratio could be 9 when hemolysis was taken as an end-point. The underlying principle in such experiments is that at the point of protection the *unbound drug* is at that concentration just insufficient to produce a particular effect. Reference to Equation (1) shows that the only way  $(X)$  can remain constant as  $P$  varies is for  $(P \cdot X)$  to vary also by exactly the same factor. This requirement can be approximately satisfied only when practically all of a drug is in the form  $(P \cdot X)$ . The case becomes one of apparent stoichiometry, falling into the category represented by the uppermost curve of Figure 7. It is difficult to see why the molar combining ratios thus obtained should have any special significance. They are certainly not to be confused with the maximal binding capacity,  $n$ . Nor is it likely that they represent a distinct class of binding centers of high affinity. As the experiments cited above show, the molar combining ratio varies with the concentration of unbound drug taken as end-point, which in turn depends upon the sensitivity of the biological indicator system. If 50 or 100 groups on an albumin molecule were capable of interacting with oleic acid and all were of identical affinity, the same molar binding ratios might be obtained under these conditions.

It should perhaps be emphasized again, in concluding this discussion on the quantitative analysis of interaction data, that all such attempts, including that presented above, are oversimplifications. The doubtful validity of some of the assumptions has been pointed out. Nevertheless these first approximations are better than none and often describe interaction phenomena accurately enough to be of real value to the investigator.

**SPECIFICITY OF INTERACTIONS** The chemical reactivities of the many plasma proteins differ as widely as their amino-acid compositions and physical properties. Drug molecules, in their interactions with these proteins, obey the same laws as do other interacting substances devoid of pharmacological activity. Any attempt to discover the nature of these laws of specificity must therefore embrace a wide variety of such substances, many of which do not fall into the drug category. Indeed, some of the most revealing investigations have dealt with constituents of normal plasma, or with organic dyes of little pharmacological interest.

Table 1 presents, in summary, the results of numerous interaction studies. In many cases the protein component was simply whole serum or plasma and no attempt was made to discover which proteins were responsible for the interaction. Many investigators, however, have sought this additional information by separation of the protein into two or more "fractions" and observing with which the substance under study specifically combined. Such data cannot be intelligently interpreted except with reference to the fractionation scheme employed. As different techniques may yield variant results a brief digression into some technical aspects of protein fractionation will be necessary.

given fraction of drug is bound in the plasma is meaningless unless qualified by an indication of the unbound drug concentration at equilibrium. If conclusions bearing upon pharmacological action are to be drawn, interaction studies must be carried out at therapeutic drug levels, for experiments at arbitrary higher levels will usually (but not necessarily) give underestimates of  $\beta$ .

(3) The smaller the dissociation constant the tighter will be the binding and the greater the fraction bound. On the other hand the equation shows that a small  $K$  can be offset by a large  $(X)$ , so that at high enough concentration essentially all of a drug will be present in unbound form. As noted above, if  $K$  is considerably smaller than  $nP$ , the whole term containing  $K$  becomes negligible. This means that all drugs with very high affinity should behave identically. Affinity differences will be obscured and fractional binding will depend upon the drug concentration alone. All such drugs will be completely bound at comparably low concentrations.

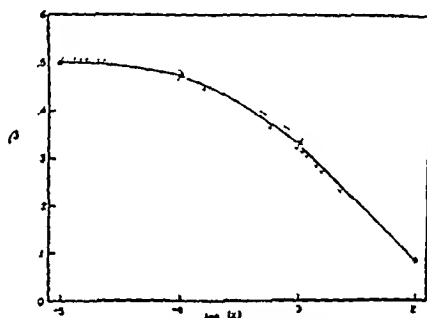


FIG. 8 For explanation see text

(4) Other things being equal, the greater the total number of receptor groups per molecule, the larger will be the fractional binding of interacting molecules.

In Figure 8 the hypothetical interaction analyzed in the previous section is plotted in terms of  $\beta$ . The curve, as was to be expected, is identical with that of Figure 7 for which  $K/nP = 1$ , and the maximal binding is 0.5. Frequently it is only possible to investigate an interaction at low drug concentrations. If, in such a case, two measurements at concentrations differing by a factor of ten give the same fractional binding, it can safely be assumed that the maximal value of  $\beta$  has been nearly reached. The system can therefore be characterized in certain important respects, and the ratio  $K/n$  obtained, even though distinct values for each constant cannot be determined. It is to be recognized that every interaction will show an unchanging, maximal value of  $\beta$  below a certain drug concentration. For example, the finding that chloromycetin is 45% bound to crystalline bovine albumin through a considerable range of concentration, reported by Smith and his associates (238) as an anomalous finding, should in fact have been expected, and could have been used to derive information of some quantitative value about the interaction.

Frequently the concentrations of protein and of total drug are varied simultaneously to determine what molar ratio gives a certain constant effect. Davis



# DRUG-PROTEIN INTERACTIONS

	B (h) B (ho) B, A, G (h)	U U Various B (h)	S A G (eu) G (thrombin)	G G (pseudo-) Other G (eu)		Low pH	tion Data on K
Calcium	G (h)	P	Caein		-	+	Tightly bound only in fraction containing thrombin about 13-16
	F (ho)	SR	Caein		+	+	Crystalline metal-combining protein 4N 2Na 1 Cu in complex
Copper	Caein		G (β <sub>1</sub> pseudo) in IV 7		(-)		(11)
	F (h)	Color reaction	S		-		(205)
	S (h)	P	A		(+)		(30, 40, 223)
	S (h)	E	B		(-)		(40, 252)
	S (h)	DA					(11)
Gold (colloidal)	S (h)	B	G (β <sub>1</sub> pseudo) in IV 7				(131, 184, 210)
Iron	F (h), S (h)	B (h)	G (β <sub>1</sub> pseudo) in IV 7				(256)
	F (h)	Color reaction	S		(+)		(11)
Magnesium	S (h)	U	B				(11)
Mercury	A* (h)	Various	A*				(11)
	S (ho)	Bo	B				(205)
Potassium	S (h)	E	0				(11)
Silver	S (h)	E	A				(11)
	S (h)	DA	B				(187)
	S (h)	DA	n				

TABLE 1

*Results of some representative interaction studies, classified according to interacting substance*

Concordant results of several investigators are often grouped together Discrepant results are listed separately Meanings of the symbols are given at end of table

SUBSTANCE	PROTEINS INVESTIGATED	METHOD	PRIMARY INTERACTION WITH	SECONDARY INTERACTIONS WITH	NO INTERACTION WITH	EASE OF REVERSIBILITY	COMPLEX DISOCLATED BY	REMARKS	REFERENCES
I INORGANIC SUBSTANCES									
Water	Various soluble proteins	A	All other soluble proteins						(32, 45, 224)
ANIONS									
Chloride	A (ho) S (ho) A* (h, h) S (d) S (h)	C E.M.F. O.P., D U U	A S A* 0 0			+	High pH	$r > 26$ $\beta < 0.6$ (0.1 M) $r = 2-5$ (0.2-12 M) (0.376%)	(187) (117) (220, 221) (104) (11)
Iodide	S (h)	U	0					(0.1%) Not done at lower concentration	(61)
(Protein-bound iodine)	F (h)	P	G ( $\alpha, \beta$ ) in IV	A and other fractions		-		Increased binding if iodide added	(4, 217)
Phosphate	S (ho) A* (h)	U EM	S A*			+		$\beta = 0.5$ (3.1 mg %) (0.1-0.8 M)	(180) (2)
Sulfate	F (h, h) S (h) S (d) S (h)	SP Renal clearance U U	A* S(?) 0 0		G (?)			(12 mg. %) ( $> 4.5$ mg %)	(22, 24) (18) (104) (118)
Thiocyanate	A* (h)	D	A*			+		$r = 11-24$ (0.2-12 M)	(221)

# DRUG-PROTEIN INTERACTIONS

	S (h)	E Sy Sh	A S A*	Bovine fraction?	G	(*) (+)	cholesterol pH > 13	r = ... Protection of ... oxidation	
Bilirubin	S (h)								(10, 50, 100)
	S (h, b)								(104, 105)
	F (h, b)								(50)
	S (h)	D, E, U, Sh	Fraction of "A" or "G"	A	G (β, γ)	α <sup>-</sup> , A <sup>+</sup>	50% acid methanol (Indirect van den Bergh)	r = 3.3, β = 80 when A* in equilibrium with solid bilirubin	(11, 97)
	F (h)	D, E, Sy	G (α) in V1	A*, G (α)					(97)
			G (pseudo-)					Some unbound (> 3 mg %)	(210)
	S (h)	Y	A		G	+			(11)
Biliverdin	S (h)	L	A		G, φ	+			(11, 97)
Coproporphyrins	S (h)	L	A*						
Hematoporphyrin	S (h)	Sy	A		G				
Urobilin	S (h)	E	A		A, G				
Uroporphyrins	S (h)	L	0						
	S (h)	E, D, B (hem)	A					Displace other compounds from A in diffusion experiments	(11)
	S (ho)	ST	B					Reduced binding in certain human sera	(149)
	S (ho)	ST, U	0					Cannot confirm Lecomte du Nohy	(255)
	S (h, ho)	ST	B					Binding increases after extracting lipids	(49)
	F (h)	B (hem)	A*					Complete binding (Cholesterol 3 mg %, ester 10 mg %) (Protein 0.9%)	(91)
		E	S					18% total sterol in precipitate of which 90% in ouglubulin, 10% which 90% in ouglubulin, 10%	(94)

TABLE 1—Continued

SUBSTANCE	PROTEINS INVESTIGATED	METHOD	PRIMARY INTERACTION WITH	SECONDARY INTERACTIONS WITH	NO INTERACTION WITH	EASE OF REVERSIBILITY	COMPLEX DISOCCUPIED BY	REMARKS	REFERENCES
II ORGANIC COMPOUNDS PRESENT IN NORMAL OR PATHOLOGICAL PLASMA									
Glucose	S (h) S (h)	U E	S O					$\beta$ = 25-33 normally Normal and diabetic sera	(60) (11)
Other sugars	F (h)	P	G ( $\alpha$ 1) in IV-6			(-)		Firmly combined glyco- and mucoproteins	(49, 78)
Indican	S (h)	E	A	G				With A (< 1 mg %) Some with G (> 1 mg %)	(12)
Lipids, various	F (h)	P	G ( $\alpha$ 1 $\beta$ 1) in III-0, IV-0			-		Firmly combined lipoproteins	(49, 78)
Melanogen	S (h)	E	A		G			Patient with melanoma	(11)
Urate	S (h, s) S (h, ch)	E, U U	A, O S			(+)	Uricosuria agents do not reduce protein binding	By electrophoresis partially bound (4.6-12 mg %) but all ultrafilters (at what concentration?) $\beta$ = .20 normally Suggests polymeric urate in plasma	(11) (151, 276-278)
Urea (or "NPN")	S (d) S (h) Blood (d) F (h, h)	U P P V	S S Blood A*			(+) + + (+)	MgSO <sub>4</sub> See remarks Various interacting anions	$\beta$ = 37 normally No evidence for claim here that polypeptides are bound NPN was measured Olate (25 mg/cc.) releases NPN normally bound to plasma protein precipitate Denaturant (6 M)	(159) (153) (214) (22, 24)
	S (h)	E	O					(148 mg %)	(11)

# DRUG-PROTEIN INTERACTIONS

III ALIPHATIC AND AROMATIC ANIONS DETERGENTS									
		LM		SP		G (γ)		G (β lacto)	
Acetate		A* (b)	A* (b)	A*	A*	Apparent stool osmotic	High pH	(0.1 M)	
Trichloroacetate		A* (b)	A* (b)	A*	A*	Apparent stool osmotic +	High pH	Mole ratios for stabilization	Quantitative
Medium chain fatty acids		I (b)	U, B (hom)	SP	A*	G (γ) Egg A	High pH	Specificity studies data	Quantitative
Oleate		Blood (d)	Blood	P	Blood	II, III, IV, Galatin	High pH	Complete binding (2.5 %)	(214)
		F (b)	B (hom) So	A*	A*	+	High pH	Mole ratios for protection	(58, 58)
								Quantitative data on K, n, ΔF	(259)
								Specificity study	(41)
								Essentially a precipitation	(194-196)
								Complex 1 = half of acid binding capacity	(70)
								Complex 2 = full acid binding capacity	
								Mole ratios for stabilization	
								Decanol crystallizes with A*	(49)
								Ethyl, amyl alcohol and acetates	(174)
								Aliphatic alcohols and esters	(16)



# DRUG-PROTEIN INTERACTIONS

Rose Bengal	F (d)	Sb	A	G	Amino acids, lipids, carbohydrate	Apparatus, stomach	cholate	Quantitative data	K, n, $\Delta V$	(100, ...)
Sulfonated dyes	F (b)	By, D	A*		G (?)	+	High pI Various other anions	Increases conductivity, (by displacing salts?)	(173, 174)	
V VOLATILE ANESTHETICS										
Chloroform	S (p)	So, VP, FP	B		Ether-extracted lipids	+		Distribution coefficient greater than in water	(183)	
Cyclopropane	Blood (b)	So	Blood		Ether-extracted lipids	+		Distribution coefficient less than in water	(114)	
Ether	S (p)	So, VP, FP	B					Distribution coefficient greater in water	(183)	
	Blood (b)	So	0					Distribution coefficient less than in water	(183)	
Ethylalco	Blood (b)	So	Blood							
Nitrous oxide	Blood (b)	So	0							
VI PHARMACOLOGICALLY ACTIVE ORGANIC ACIDS										
							(+)	Some unbound (therapeutic concentrations) Only D-lipal to G at high concentrations	(11)	
							+	Specificity studies 17 compounds	(100)	
							+	$\beta = 68$ (3.8 mg %) and 50 (13.4 mg %)	(77)	
							+	Displaces phosphatide of an active lipoprotein in clotting mechanism (?)	(27 38 30 44, 88 133 135, 197 274, 275, 280)	
Barbiturates	S (b)	E	A							
	A* (h b)	U	A*							
		D	A							

TABLE 1—Continued

SUBSTANCE	PROTEINS INVESTIGATED	METHOD	PRIMARY INTERACTION WITH	SECONDARY INTERACTIONS WITH	NO INTERACTION WITH	EFFECT OF REVERSIBILITY	COMPLEX DISOCLATED BY	REMARKS	REFERENCES
III ALIPHATIC AND AROMATIC ANIONS DETERGENTS ALIPHATIC ALCOHOLS—Continued									
Substituted phenols	A* (b)	D	A*				High pH	Quantitative data on K, n, $\Delta F$ Specificity study Mole ratios for stabilization	(259)
	A* (b, b)	SP	A*			+	Apparent solubility		(70)
	S (bo, b)	U	S					Tetrachlor o-biphenol Antiseptic action also reduced in serum	(6, 7)
	S (b)	E	A, G (r)					Phenols and cresols in uremic serum	(11)
	S (tr)	D	S			+		Binds guaiacol, xylol, toluene, which displace other compounds	(14)
V DYES									
Most dyes	F (various)	Various	A*					Only those of special interest listed below Dye interaction studies indexed in Table Ia	
Basic dyes, various	S (b)	E	A	G (r)		(+)			(11)
Colloidal dyes, various	S (bo)	P	"G(eu)"			"—"		Few data and no proof Cf (21)	(67)
Evans Blue	F (b)	E	A	G(e)	G(r)	+			(201)
Phenol Red	S (bo)	U	S			(+)	0.35% phenol (302) Hip-puran di-oxast (239) Oleate (214)		See Table Ia
Prontosil	S (b)	E	A		G	+		Complete binding (1.5 mg %) Some unbound (62.6 mg %)	(11 12 225)



	F (b)	A	G	G		concentration	concentration
Aminocyclitine (Quinacrine)	F (b)	?	A			Some unbound (0 mg %)	(218)
	S (h)	E	A			Complete binding (0.4 %)	(14, 246)
Aminopyrine	S (ho)	U	B		?	Bound much less than pilocarpine	(186)
Atropine	S (b, r)	D, B (c g)	B		+		(14)
	S (ho)	D	B		(+)	No data on concentration	(125, 254)
Caffeine	S (various)	D	0			$\beta = .50 - .90$ (therapeutic concentration)	(14, 186)
Choline	F (b)	?	A*		(+)	Dialysis from S (b), not from S (r) (0.25%)	(35, 344)
Cinchona alkaloids	S (b, ho, r)	D	B		?		(3)
Cocaine	A (b, o, g)	E, SP	A			Protected against oxidation	(14)
Doxors ribose-nucleate	S (h)	Sb	B			No data on concentration	(22, 24)
Epinephrine	S (various)	D	0	G (r)		Denaturant (3.5 M)	(14)
Guanidine	F (b, b)	V	A*			No data on concentration	(260)
Histamine	S (various)	D	0		+	High pH (?), $\alpha$ naphtha	(14, 248)
Indole acetate (auxin)	A* (b)	D	A			less-acetate, indole - butyrate	(15, 16, 186, 245-248)
Morphine	S (b, b, r, s)	D	B		+	Citrate, peptide	
						Atropine, other 0.1%, chloroform 0.05%	

TABLE 1—Continued

SUBSTANCE	PROTEINS INVESTIGATED	METHOD	PRIMARY INTERACTION WITH	SECONDARY INTERACTIONS WITH	NO INTERACTION WITH	EASE OF REVERSIBILITY	COMPLEX DISO- CIATED BY	REMARKS	REFERENCES
VI PHARMACOLOGICALLY ACTIVE ORGANIC ACIDS—Continued									
Iodine - contain- ing acids	A (h)	U	A			(+)	Hippuran and diatrizoate dis- place phenol red	Skidan $\beta = 18$ , hippuran $\beta = 70$ (1 mg %, 4 % A)	(239)
	B (hc)	U	B			-		No supporting data (What con- centration?)	(84)
	B (h)	P	B					Priodax persists in vivo and firmly bound to protein precipitate	(265, 266)
p-amino-hippu- rate	B (h)	Renal clear- ance	B			?		$\beta = 17$ (25-100 mg %)	(236)
p-amino-salicyl- ate	B (h), A (b)	U	A			+		$\beta = 60$ (10 mg %, 3.5 % A)	(266)
Salicylate	B (h, b)	D	B			+		$\beta \approx 0.50$ (30 mg %) Hypersens- itive patients bind less	(250)
	B (h)	P	B					$\beta = 75$ (10 mg %) and 50 (20 mg %)	(93)
	B (h)	U	B					Completely bound (45 mg %), some unbound (180 mg %)	(237)
	B (h)	E, U	A						(11)
Salicylurate	B (h)	D	B					Complete binding (20 mg %)	(251)
VII ALKALOIDS AND RELATED COMPOUNDS									
Acetylcholine	F (h) A (d, p)	EA B (f)	G(c) in IV 6 "A"					Plasma cholinesterase Destruction not ruled out	(103) (119)
Acetyl trypto- phane	A* (h, b)	SP	A*			+	High pH		(25)
Adenine Adenosine Adenylic acid	F (b)	D	A*		G (r)	+		Quantitative data, $\Delta F$	(141)

F (h)	E	A	G	?	Citrate, peptide	Complete binding (0.4 %)	(14, 246)
Aminocyclitine (Quinacrine)	U	S		+		Bound much less than pilocarpine	(186)
Aminopyridine	D, B (c g)	S		(+)		No data on concentration	(14)
Atropine	D	S				$\beta = 50-90$ (therapeutic concentration)	(125, 234)
Caffeine	D	0				Dialyzes from S (h), not from S (r) (0.25%)	(14, 186)
Choline	?	A*		(+)	High pH with other		(35, 244)
Cinchona alkaloids	D	S		?		Protected against oxidation	(3)
Cocaine	E, SP	A				No data on concentration	(14)
Deoxyribosuccinate	S <sup>b</sup>	S			Various in reacting anions	Denaturant (2.5 M)	(22, 24)
Ephedrine	D	0	G (r)			No data on concentration	(14)
Guanidine	Y	A*					(260)
Histamine	D	0		+	High pH (?), $\alpha$ naphthalene, benzo-acetate, indole, tyrosine		(14, 248)
Indole acetate (auxin)	D	A					
Morphine	S (h, b, r, s)	S		+	Citrate, peptide	$\beta = 95-29$ (0.2-2.0 mg %)	(15, 16, 186, 245-248)
Pilocarpine	S (ho, r, h, s)	D, B (c g)		+	Atropine, other 0.1%, chloroform 0.05%		

TABLE 1—Continued

SUBSTANCE	PROTEINS INVESTIGATED	METHOD	PRIMARY INTERACTION WITH	SECONDARY INTERACTIONS WITH	NO INTERACTION WITH	EASE OF REVERSIBILITY	COMPLEX DISOCLATED BY	REMARKS	REFERENCES
VII. ALKALOIDS AND RELATED COMPOUNDS—Continued									
Procaine	S (various)	D	S			+	Various substances		(14)
Prothigmine	F (h), S (h)	EA, SP	G (a) in IV-6			+	Acetylcholine	Protect plasma cholinesterase against DFP denaturation	(102, 144)
Carbamoyl choline									
VIII. CARDIAC GLYCOSIDES									
Digitoxin and various other glycosides and aglycones	A (h), F (r) S (f, r, o) S (b, r, o) S (h) S, F (various) F (h)	D, P B (f h, r h) U, B (f h) B (f h) D, B (f h) B (f h)	A S S A A*		G (gu) (pseudo)  Egg A lecithin cholesterol All G fractions I, II + III, IV 1, IV-4	?	pH > 8.3 saponin, bile salts	Digitoxin $\beta$ = 50 (1 mg %, 1% A (h))  Saponin bile salts, high pH do not displace	(112, 113) (217) (150) (152) (85, 86) (215)
Strophanthus glycosides and aglycones	A (h), F (r) S (b, r, o) S (h) S, F (various) F (h)	D, P U, B (f h) B (f h) D, B (f h) B (f h)	A S O O O		G (gu) (pseudo)	+	Ethanol		(112, 113) (150) (152) (85, 86) (215)
IX. MISCELLANEOUS CHEMOTHERAPEUTICS									
Arphenamine	S (r)	P, SP	O	A		?	Low pH	Also interacts with gum arabic	(179)

Blennithyl Tar trates	S (b)	U	S		G (pseudo-), A	+	High pH	Mono-blennithyl tartrate $\beta = 40$ di and tri blennith tartrate $\beta = 94 - 96$	(228) (5)
Diamidines	S (r)	B (bac.)	S			?		Serum protects against antibac- terial action	(92)
Naphthoquinones	F (b) F (h), S (var- ious)	D, So D, So	A* A*	Other frac- tions ex- cept II	G (r) (II) G (b, r)	(+) $\pm$ ?	Low pH Caprylate	Specificity studies Interactions with A* poorly re- versible, other interactions easily reversible	(30, 31) (124) (46)
Neurephen- amine	S (h)	E	A		G			Complete binding (40 mg %), some unbound (160 mg %)	(11)
Buramin (Bayer 205)	S (various) S (ho, r) S (h)	SP P, U P	B "A" fraction S, G (r)	Casidin, A(r)	G fraction +	- -		Protects (6-10%) sera of 10 species against coagulation Smaller hydrolysis products less bound	(143) (155) (20, 86, 241)

X. ANTIBACTERIAL CHEMOTHERAPEUTICS AND RELATED COMPOUNDS

Chloromycetin	A* (b)	D	A*			(+)		$\beta = 45 - 60$ (therapeutic concen- trations)	(238)
p-aminobenzoate	S (h)	U	S			+		$\beta = 20 - 30$ (1-3 mg %)	(157)
Penicillins	F (b, b) Various	D Various	A* A*		All other fractions Other frac- tions	+		Quantitative data $\beta$ (0.1 $\gamma$ /cc) from 56 for benzyl (G) to 96 for n heptyl (K)	(231) (17, 71-73, 75, 142, 233, 258, 261)
Streptomycin	S (b) S (h)	? B (bac.)	0 S			?		Loss of potency in serum resem- bling a slow interaction, not de- struction	(202) (122)

TABLE 1—Continued

X ANTIBACTERIAL CHEMOTHERAPEUTICS AND RELATED COMPOUNDS—Continued									
SUBSTANCE	PROTEINS INVESTIGATED	METHOD	PRIMARY INTERACTION WITH	SECONDARY INTERACTIONS WITH	NO INTERACTION WITH	EASE OF REVERSIBILITY	COMPLEX DISSOCIATED BY	REMARKS	REFERENCES
Streptomycin	A* (b)	D, B (bac.)	0 or A*					Bacteria protected by A* (0.05%) from streptomycin (0.5 mg %) but no binding by dialysis	(142)
Sulfonamides	A* (b) Various	D Various	A* A*		Other fractions	+ +	PABA and other anions	Specificity study of substituted sulfadiazines 41 compounds $\beta$ (10 mg %) from .20 for sulfanilamids to .93 for sulfamethylthiadiazole	(264) (1, 54, 55, 64, 65, 76, 98, 120, 157, 185)
Sulfones	A* (b)	So	A*					4 - amino - 4' - $\beta$ - ethoxyethyl - aminodiphenyl sulfone	(132)
"Proteins Investigated" S serum (or whole plasma) F -									

"Proteins Investigated" S serum (or whole plasma), F, plasma fractions, A, albumin fraction (impure), A\*, crystalline albumin.

(b) bovine (c) cat (ch) chicken, (d) dog (f) frog, (g) goose, (ho) horse, (h) human, (p) pig (r) rabbit, (s) sheep

"Methods" (see pp 104-111) B, biological action, C, conductivity, D, dialysis, DA, differential adsorption, Di, diffusion, E, electrophoresis, EA, enzyme activity, EM, electrophoretic mobility, FP, freezing point, OP, osmotic pressure, P, precipitation, SB, stabilization (of drug), So, solubility, SR, sedimentation rate, ST, surface tension, Sg, spectrophotometry, U, ultrafiltration, V, viscosity, VP, vapor pressure

(bac.) bacteria, (c) cat, (d) clotting time (f) frog heart, (hem) hemolysis, (m.a.) mouse assay, (r) rabbit heart

"Primary, secondary, no interaction with" Under "primary interaction" is listed that of highest affinity—i.e., the interaction observed at lowest drug concentration. Under "secondary interaction" are listed others simultaneously demonstrable at higher drug concentration. Under "no interaction with" are listed only those instances in which adequate evidence allows certain proteins to be ruled out—i.e., no interaction observed at any reasonable concentration of drug.

0 none S, interaction, in an experiment with serum, where the specific protein interacting was not further identified, A, A\*, interaction, with albumin (impure) or crystalline albumin, G, interaction, with globulin fraction, further specified as to ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) and Harvard fraction if data available

"Ease of Reversibility" +, definitely reversible by simple means, e.g., dilution, (+), probably reversible by simple means, not proved definitely, —, apparently not reversible by simple means.

"Complex dissociated by" Lists special reagents which seem to dissociate the complex without denaturation of the protein.

"Remarks" Pertinent data and conditions of experiments. As in text  $\beta$  = fraction of total drug bound at the stated concentration, r = moles bound drug per mole total protein under the experimental conditions, n = maximum value of r, at infinite drug concentration.

A general discussion of recent advances in the fractionation and characterization of the plasma proteins is obviously beyond the scope of this paper. A brief résumé, based largely upon recent reviews by Edsall (78), Cohn (49), and Gibson (96), will suffice to present the facts that are pertinent to a discussion of interaction specificity.

Human plasma is a circulating fluid tissue of some 3000 cc volume, containing about 7 per cent (or 200 grams) of protein. Over twenty-five distinct proteins

TABLE 1a

*References to interactions of dyes with plasma proteins*

#### AZO DYES

Amaranth (140)  
Azorubin (11)  
Azosulfathiazole (138, 139)  
Azo Violet (11)  
Biebrich Scarlet (200)  
Brilliant Congo Red (11, 127)  
Brilliant Vital Red (68)  
Butter Yellow (12)  
Congo Red (11, 81, 107)  
Diamine Blue (67)  
Diamine Red (67)  
Evans Blue (68, 107, 108, 201)  
Helgoland Yellow (11)  
Methyl Orange (11, 138-140)  
Niagara Sky Blue (107, 201)  
Niagara Sky Blue 6 B (107, 201)  
o-amino-azo-toluol (12)  
Orange I and II (139)  
Salmon Red (11)  
Tropaeolin O (11, 200)  
Trypan Blue (62, 227, 231)  
Trypan Red (11, 62)

Eosin (11, 123)  
Erythrosin (123)  
Fluorescein (33, 61, 62, 123)  
Mercurochrome (126)  
Phenolphthalein (123)  
Phenol Red (PSP) (62, 101, 109, 123, 161, 162, 192, 202, 208, 212, 229, 239)  
Phloxin Red (123)  
Rhodamin (11)  
Rose Bengal (11, 123, 211-213)  
Tetrachlorophenolphthalein (123)  
Tetrabromphenolphthalein (123)  
Thymol Blue (123)

#### TRIPHENYLMETHANE DYES

Acid Green (St Denis) (67)  
Acid Violet (St Denis) (67)  
Basic Fuchsin (126)  
Brilliant Green (126)  
Crystal Violet (126)  
Gentian Violet (67)  
Malachite Green (67, 126)  
Patent Blue (11)  
Victoria Blue (67)

#### NITRO DYES

Naphthol Yellow (11)  
Picric Acid (259)

#### PHTHALEIN DYES

Bromphenol Blue (123)  
Bromsulfalein (26, 212)  
Bromthymol Blue (123)

#### MISCELLANEOUS DYES

Acridine Orange (67)  
Indigo Carmine (62, 193, 202)  
Lithium Carmine (62)  
Methylene Blue (6)  
Neutral Red (67)

are represented, differing in size, shape, composition, charge, solubility and chemical reactivity. They can be separated into groups ("fractions") by methods that are sensitive to one or another of the differences in physical properties. As the pattern of similarities and differences is rather complex, those proteins falling into a given fraction in one method may be dispersed among several fractions in another.

In the order of increasing solubility (*e g*, in ammonium sulfate), the usual categories are *fibrinogen*, *eu-globulin*, *pseudo-globulin*, *albumin*. As the molarity of

salt chosen as boundary between one fraction and the next is rather arbitrary, all fractions obtained in this way are more or less contaminated with proteins of neighboring fractions. For example, the fraction precipitating between half- and fully-saturated ammonium sulfate consists of several albumins, and also contains certain globulins of similar solubility. If a substance interacts specifically with one of the latter it would naturally be reported to combine with "albumin". A number of such instances can be found in Table 1, where subsequent study with purer proteins served to rule out albumin and implicate a particular globulin.

In order of decreasing rate of migration toward the anode in an electric field, the categories are albumin,  $\alpha_1$ - and  $\alpha_2$ -globulin,  $\beta_1$ - and  $\beta_2$ -globulin, fibrinogen,  $\gamma_1$ - and  $\gamma_2$ -globulin. Crude electrophoretic apparatus might only permit two fractions to be distinguished—rapidly migrating "albumin" and slowly migrating "globulin". Each component distinguished by electrophoresis contains proteins that happen to migrate at the same rate, but these need not share the same solubility properties. A substance migrating strictly with albumin in an electric field might be a pseudo-globulin in salt precipitation. Bilirubin was observed by Bennhold (11) to migrate with albumin in crude electrophoresis. Bendien and Snapper (10) had earlier reported that a portion of the bilirubin interacts with certain globulins that are retained by a partially permeable ultrafilter. The apparent anomalies in this typical case appear to be resolved by the recent discovery of a tightly-bound bilirubin-protein complex that precipitates as a pseudo-globulin and migrates as an  $\alpha_1$ -globulin on electrophoresis (49). Evidently similar considerations apply to all other fractionation methods (*e.g.*, ultracentrifugation) short of complete purification of homogeneous proteins.

The low-salt, low-temperature, ethanol method of fractionation represents a refinement of earlier solubility techniques, which has already yielded several products of high purity, including fibrinogen, crystalline albumin, and a metal-combining  $\beta_1$ -globulin. Preparations of  $\gamma$ -globulins have also been produced but as numerous specific antibodies are represented it must be assumed that, at least from the standpoint of surface configurations, a variety of proteins is present.

The remaining fractions are still mixtures but the components of each have been characterized in some detail. The fractionation method and the full description of each fraction are to be found in Edsall's review (78) and the earlier reports of Cohn and his associates (47, 48). To clarify those entries in Table 1 that refer to the Harvard fractions, the nomenclature is set forth in condensed form in Table 2.

**Fibrinogen.** The principal interaction of this protein with thrombin, in the complex clotting mechanism, is evidently in the class of protein-protein interactions, which will not be discussed here. No specific reactivity of fibrinogen with small molecules has been demonstrated, although certain substances are bound at concentrations higher than those required to demonstrate a primary interaction with another protein. Certain substituted naphthoquinones, for example, interact with most fractions including fibrinogen, but the complexes with the latter are appreciably looser than with the others (124). Methylene blue is bound by fibrinogen, to a lesser degree than by most other fractions (103). Fibrin films bind



a number of dyes (233) but it has not been demonstrated whether soluble fibrinogen would also react with them. The insignificance of interactions with fibrinogen is further indicated by the observation that the same interaction data are generally obtained in serum and in plasma

*$\gamma$ -globulins* The  $\gamma$ -globulins appear to be involved almost exclusively in those highly specific protein-protein interactions known as immune reactions. Hydrogen ion, certain alkali-earth cations (69, 106), and various denaturants evidently react with all proteins, and the  $\gamma$ -globulins (and fibrinogen) are no exception. As in the case of fibrinogen, various substances that interact with more than one protein may be bound to  $\gamma$ -globulin at high concentration. But these are hardly to be considered specific interactions. The thermal denaturation of  $\gamma$ -globulin is slowed by sucrose and glycine (46) but these are the only reported instances of interaction with small molecules. It must be concluded that these proteins are

TABLE 2  
*Nomenclature of the Harvard fractions\**

FRACTION	MAJOR COMPONENTS
I	Fibrinogen (175)
II	$\gamma$ globulins (180)
III-0	$\beta_1$ -lipoprotein (180)
III-1, 2, 3	Isoagglutinins, thrombin, plasmin, complement (C') (180)
IV-1, IV-4†	Various $\alpha$ - and $\beta$ -globulins, including an $\alpha_1$ -lipoprotein, $\alpha_2$ -glycoproteins, and several enzymes (252)
IV-7	Metal-combining $\beta_1$ -globulin (199a, 251a)
V-1	Bilirubin-containing $\alpha_1$ -globulin
V	Albumin (with about 3% globulin impurity)
Crystalline albumin	

\* After Cohn (49)

† IV-2, 3 and IV-5, 6, 7 are subfractions of IV-1 and IV-4, respectively

endowed with a low order of chemical reactivity, as usually understood, and that the forces responsible for combination with antigens are somehow unique. Configurational factors and surface charge distribution presumably play a role (188) but the intimate mechanisms are still poorly understood.

*$\alpha$ - and  $\beta$ -globulins.* Purification of most of the  $\alpha$ - and  $\beta$ -globulins is far from complete but a characteristic pattern of interaction specificity is beginning to emerge. Most of the members of this group are either enzymes, or occur in the plasma as conjugated proteins carrying smaller molecules in close association.

The enzymes include thrombin, plasmin, several peptidases, amylase, lipase, alkaline phosphatase and choline esterase. The specificity of their interactions finds expression in differing affinities for various substrates and inhibitors. The plasma cholinesterase, which proves to be an  $\alpha$ -globulin appearing in fraction IV-6, has a specific affinity for choline esters as substrates and for carbamates of quaternary ammonium compounds related to choline as inhibitors (102). Interaction with the inhibitors has been demonstrated not only by the classical methods

of enzymology but also by their ability to protect the enzyme against a specific denaturant (144) The stabilizing action of plasma albumin against heat denaturation of this enzyme (102) is probably to be attributed to protein-protein interaction In the same category would be the diastase-albumin interaction, demonstrated by electrophoresis (12) Each of the other enzyme globulins has its own pattern of substrate and inhibitor specificity reflecting, on the whole, rather precise requirements in molecular structure of the interacting compounds It is not improbable that some of these enzymes combine with metallic or other prosthetic groups and there seems little reason to place such interactions in a special category

Several pigment proteins have been obtained A blue-green  $\alpha$ -globulin is found in fraction IV-2 (49) Fraction III-0 contains a blue pigment that may be hemocuprein and a pale yellow hematin-protein conjugate, neither of which is associated intimately with the lipoproteins (181) Fraction V-1 contains a stable complex of bilirubin with an  $\alpha$ -globulin (49) Bilirubin also combines with albumin but whereas the albumin complex is easily reversible (165), the globulin compound cannot be dissociated by prolonged and repeated dialysis (103) This difference in ease of reversibility was first observed by Hoover and Blankenhorn (130) who found that the serum of patients with bilirubinuria ("obstructive jaundice") yielded bilirubin in a dialysate while that of other jaundiced individuals ("hemolytic jaundice") yielded none Nevertheless all the pigment in either case can be extracted with acid 50% methanol in the indirect van den Bergh reaction (50, 160) It also appears likely, as pointed out by Bennhold (11), that the non-dialyzable form of bilirubin can be excreted by the liver, implying that the organ can split the complex, but this supposition has not been directly verified

The metal-combining  $\beta_1$ -globulin in fraction IV-7 forms complexes with iron, copper, and probably zinc, and evidently performs an important physiological function in iron transport (36, 96) The equilibria are shifted toward increased binding by alkaline conditions (49) Macheboeuf (158) has proposed an interesting model in explanation of the observed molar combining ratio in the copper-protein interaction

Fraction IV-6 contains two or more glycoproteins whose properties had long been under discussion The carbohydrates, which include hexoseamine and other sugars, are firmly conjugated to the globulin moieties (171, 252)

Finally there are at least two lipoproteins, a large  $\beta_1$ -globulin in fraction III-0 and a smaller  $\alpha_1$ -globulin in fraction IV-1 (78, 180) These are found to contain 75% and 35% lipid, respectively, including steroids, phospholipids, and other lipid-soluble molecules occurring normally in the plasma (49) At least a portion of these are not readily extracted by ordinary lipid solvents, presumably because they are conjugated firmly to the carrier globulins (40) Cholesterol and its esters are found in reversible combination with these lipoproteins (28, 49, 170) and the specific interaction of plasma estrogen (estriol) with fraction III-0 is also easily reversible (19, 207) The carotenoid precursors of vitamin A are associated with the  $\beta_1$ -lipoprotein (11, 181) but the interaction of the vitamin itself has not been studied It can be assumed that steroid hormones other than estriol will be found to interact with these same globulins

There remain a group of compounds whose interactions with globulins have not yet been fully analyzed. Shubert (226) studied various vitamins by a crude electrophoretic technique and concluded that riboflavin interacted primarily with globulin (which proved to be a eu-globulin by solubility behavior). Thiamine, on the other hand, displayed no affinity for either albumin or globulin, but its oxidation product, thiochrome, migrated with eu-globulin. Bennhold (12) reported that nicotinamide migrated primarily with globulin, and at higher concentration also with albumin. It is interesting to note that thiamine, which behaved so differently from the other vitamins, was studied at unphysiologically high concentration. It is at least possible that further study might reveal an interaction with globulin at low concentrations. In view of the known role of these substances as enzyme prosthetic groups it would seem reasonable for them all to interact with globulins.

Moore and Roaf (173, 174) showed that the volatile anesthetics ether and chloroform were more soluble in plasma than in saline. Aware of the possibility that plasma lipids might be responsible, they demonstrated the integrity of the interaction in "lipid-extracted" plasma, and consequently attributed the phenomenon to protein interaction. It now seems evident that some lipoprotein remained intact in these experiments, and furthermore, the smallest molar combining ratio that can be calculated from their data on chloroform is still absurdly large. These facts and the high lipoid-solubility of the volatile anesthetics make it appear probable that physical solution in the lipid component of the lipoproteins is responsible, rather than a specific protein interaction. Haggard (114), subsequently, could not confirm the above findings with respect to ether, in whole blood. But the same considerations probably apply in the case of ethylene and cyclopropane whose solubilities are definitely increased in the presence of plasma (183). The question could be settled by studies with purified proteins but this has not yet been attempted.

As reference to Table 1 will show, remarkably few drugs display a primary affinity for globulins. It has been claimed that a number of colloidal dyes are precipitated preferentially with globulins (67). However, some of the same dyes have been shown repeatedly to interact with albumin, and the validity of the experiments can be questioned on other grounds (21). Thorotrast (colloidal thorium dioxide) migrates exclusively with globulin (11) and displays a peculiar affinity for the reticulo-endothelial cells in the body. Colloidal gold and silver salts, which also are taken up by the reticulo-endothelial system, are bound to plasma protein (265) but it has not yet been established whether the globulins are responsible. All three bismuthyl tartrates interact exclusively with eu-globulins (5). Arsphenamine is something of a border-line case which might lead to further understanding of the minimal requirements for interaction with globulins. It is bound primarily to globulin, but also to albumin (179), while the closely related neoarsphenamine interacts only with albumin (11). The very large aromatic polypeptide trypanocide, suramin (Bayer 205), is tightly bound by the plasma proteins (143), and the evidence favors primary interaction with globulins (20, 66, 241).

In summary, the  $\alpha$ - and  $\beta$ -globulins of the plasma interact as enzymes with

of enzymology but also by their ability to protect the enzyme against a specific denaturant (144). The stabilizing action of plasma albumin against heat denaturation of this enzyme (102) is probably to be attributed to protein-protein interaction. In the same category would be the diastase-albumin interaction, demonstrated by electrophoresis (12). Each of the other enzyme globulins has its own pattern of substrate and inhibitor specificity reflecting, on the whole, rather precise requirements in molecular structure of the interacting compounds. It is not improbable that some of these enzymes combine with metallic or other prosthetic groups and there seems little reason to place such interactions in a special category.

Several pigment proteins have been obtained. A blue-green  $\alpha$ -globulin is found in fraction IV-2 (49). Fraction III-0 contains a blue pigment that may be hemocuprein and a pale yellow hematin-protein conjugate, neither of which is associated intimately with the lipoproteins (181). Fraction V-1 contains a stable complex of bilirubin with an  $\alpha$ -globulin (49). Bilirubin also combines with albumin but whereas the albumin complex is easily reversible (165), the globulin compound cannot be dissociated by prolonged and repeated dialysis (103). This difference in ease of reversibility was first observed by Hoover and Blankenhorn (130) who found that the serum of patients with bilirubinuria ("obstructive jaundice") yielded bilirubin in a dialysate while that of other jaundiced individuals ("hemolytic jaundice") yielded none. Nevertheless all the pigment in either case can be extracted with acid 50% methanol in the indirect van den Bergh reaction (50, 160). It also appears likely, as pointed out by Bennhold (11), that the non-dialyzable form of bilirubin can be excreted by the liver, implying that the organ can split the complex, but this supposition has not been directly verified.

The metal-combining  $\beta_1$ -globulin in fraction IV-7 forms complexes with iron, copper, and probably zinc, and evidently performs an important physiological function in iron transport (36, 96). The equilibria are shifted toward increased binding by alkaline conditions (49). Macheboeuf (158) has proposed an interesting model in explanation of the observed molar combining ratio in the copper-protein interaction.

Fraction IV-6 contains two or more glycoproteins whose properties had long been under discussion. The carbohydrates, which include hexoseamine and other sugars, are firmly conjugated to the globulin moieties (171, 252).

Finally there are at least two lipoproteins, a large  $\beta_1$ -globulin in fraction III-0 and a smaller  $\alpha_1$ -globulin in fraction IV-1 (78, 180). These are found to contain 75% and 35% lipid, respectively, including steroids, phospholipids, and other lipid-soluble molecules occurring normally in the plasma (49). At least a portion of these are not readily extracted by ordinary lipid solvents, presumably because they are conjugated firmly to the carrier globulins (40). Cholesterol and its esters are found in reversible combination with these lipoproteins (28, 49, 170) and the specific interaction of plasma estrogen (estriol) with fraction III-0 is also easily reversible (19, 207). The carotenoid precursors of vitamin A are associated with the  $\beta_1$ -lipoprotein (11, 181) but the interaction of the vitamin itself has not been studied. It can be assumed that steroid hormones other than estriol will be found to interact with these same globulins.

There remain a group of compounds whose interactions with globulins have not yet been fully analyzed. Shubert (226) studied various vitamins by a crude electrophoretic technique and concluded that riboflavin interacted primarily with globulin (which proved to be a eu-globulin by solubility behavior). Thiamine, on the other hand, displayed no affinity for either albumin or globulin, but its oxidation product, thiochrome, migrated with eu-globulin. Bennhold (12) reported that nicotinamide migrated primarily with globulin, and at higher concentration also with albumin. It is interesting to note that thiamine, which behaved so differently from the other vitamins, was studied at unphysiologically high concentration. It is at least possible that further study might reveal an interaction with globulin at low concentrations. In view of the known role of these substances as enzyme prosthetic groups it would seem reasonable for them all to interact with globulins.

Moore and Roaf (173, 174) showed that the volatile anesthetics ether and chloroform were more soluble in plasma than in saline. Aware of the possibility that plasma lipids might be responsible, they demonstrated the integrity of the interaction in "lipid-extracted" plasma, and consequently attributed the phenomenon to protein interaction. It now seems evident that some lipoprotein remained intact in these experiments, and furthermore, the smallest molar combining ratio that can be calculated from their data on chloroform is still absurdly large. These facts and the high lipid-solubility of the volatile anesthetics make it appear probable that physical solution in the lipid component of the lipoproteins is responsible, rather than a specific protein interaction. Haggard (114), subsequently, could not confirm the above findings with respect to ether, in whole blood. But the same considerations probably apply in the case of ethylene and cyclopropane whose solubilities are definitely increased in the presence of plasma (183). The question could be settled by studies with purified proteins but this has not yet been attempted.

As reference to Table 1 will show, remarkably few drugs display a primary affinity for globulins. It has been claimed that a number of colloidal dyes are precipitated preferentially with globulins (67). However, some of the same dyes have been shown repeatedly to interact with albumin, and the validity of the experiments can be questioned on other grounds (21). Thorotrast (colloidal thorium dioxide) migrates exclusively with globulin (11) and displays a peculiar affinity for the reticulo-endothelial cells in the body. Colloidal gold and silver salts, which also are taken up by the reticulo-endothelial system, are bound to plasma protein (265) but it has not yet been established whether the globulins are responsible. All three bismuthyl tartrates interact exclusively with eu-globulins (5). Arsphenamine is something of a border-line case which might lead to further understanding of the minimal requirements for interaction with globulins. It is bound primarily to globulin, but also to albumin (179), while the closely related neoarsphenamine interacts only with albumin (11). The very large aromatic polypeptide trypanocide, suramin (Bayer 205), is tightly bound by the plasma proteins (143), and the evidence favors primary interaction with globulins (20, 66, 241).

In summary, the  $\alpha$ - and  $\beta$ -globulins of the plasma interact as enzymes with

certain small molecules meeting a rigid specificity requirement, and as carrier proteins in a number of tight complexes with diverse normal plasma constituents. Certain water-insoluble compounds of physiological importance are bound more or less reversibly in fractions containing lipoprotein. The functional value of such arrangements in the transport of insoluble substances has been discussed at length by Bennhold (11, 12), Rothlin (216) and others (57). The very few drugs that have been proved to interact with plasma globulins contain heavy metals or are poorly soluble in water, or both.

**Albumin** That the plasma albumin shows the greatest diversity of all the proteins in its capacity for interaction should be apparent from a cursory inspection of Table 1. A phenomenal array of substances foreign to the body, whether endowed with pharmacological activity or not, interacts with plasma proteins and (with few exceptions) all those that have been adequately studied prove to combine with albumin.<sup>3</sup> To what common features, then, do so many different compounds owe their reactivity toward this protein and what molecular properties enhance or diminish affinity for albumin?

Titration data provide an indication of the total acid- and base-combining capacity of plasma albumin and the actual numbers of ionic groups in the charged state at any pH (45, 224). These data are reasonably well confirmed by amino acid analysis, on the assumption that the sum of the diamino acids (lysine and arginine), histidine, and the terminal amino groups is equal to the potential number of cationic residues, and similarly that the sum of the dicarboxylic acids (glutamic and aspartic), tyrosine, the terminal carboxyl groups, and possibly cysteine, is equal to the potential number of anionic residues (after correction for amide-covered carboxyl groups). This leads to a figure of 109 cationic and 120 anionic residues, assuming a molecular weight of 69,000 (78).

The addition or removal of hydrogen ion is an instructive exercise because a titration is the elemental form of interaction experiment, yielding groups of different affinity (pK) as the pH is varied. The unique feature of a titration is the range of concentration (of  $H^+$ ) explored—a range that cannot be duplicated with any other interacting molecule. Very likely all ionic interactions are to be regarded as competitions with hydrogen ion for the particular protein groups concerned.

The isoelectric point of albumin is at pH 4.9 so that at pH 7.3 the molecule carries a net negative charge. The early supposition that only cations would interact under these circumstances was soon shown to be incorrect. Ionic combination between groups of opposite charge occurs irrespective of the net charge on the protein, and a great many more anions than cations have actually been proved to interact at physiological pH. Some of these ionic combinations will now be reviewed from the standpoint of interaction specificity.

Interaction of the lower fatty acids ( $C_2$ – $C_{10}$ ) is clearly favored by increasing length of the hydrophobic carbon chain (2, 23, 25). This is not a function of acid

<sup>3</sup> For the purpose of this discussion the evidence for the existence of more than one albumin will be disregarded.

strength, which differs but slightly among the compounds investigated. The primary bond is presumably electrostatic but the resulting complex is probably stabilized by van der Waal forces through close approximation of the nonpolar residue to similar portions of the adjacent protein surface. The exceedingly strong binding of oleic acid (56, 58) is entirely consistent with this picture.

Penicillin interactions allow an extension of the same principle. These compounds may be regarded as modified fatty acids containing a peculiar cyclic dipeptide as part of the long chain (59). Penicillin K with the longest aliphatic chain (n-heptyl) shows the greatest affinity, followed, in turn, by dihydro-F (n-amyl), G (benzyl) and X (p-hydroxybenzyl) (261). The difference between X and G can be attributed to the polar group of the former which is presumably attracted away from the protein toward the water phase.

The barbiturates are also substituted carboxylic acids with an intervening ring between the anionic and substituent groups. Ultrafiltration studies (100) show that binding again increases with chain length, in the order barbital < ipral < neonal < pentobarbital = amytal < oral. Compounds with cyclic side-chains, like phenobarbital, phanodorn, cyclopal and evipal all have about the same affinity as neonal. Allyl substituents confer somewhat greater affinity than the corresponding saturated groups and thiobarbiturates are bound much more firmly than their oxy-analogues.

Binding of sulfonamides to albumin increases in the order sulfanilamide = sulfacetimide < sulfapyridine < sulfadiazine < sulfathiazole < sulfamerazine = sulfamethazine, when the drugs are compared at the same concentration (10 mg %) (54, 55, 98). As each of the acetyl conjugates of these drugs is bound to the same degree as the parent compound (64, 65, 98), it may be inferred that the p-amino group is not involved in the interaction. This is in striking contrast to its role in antibacterial action, for acetylation abolishes or markedly diminishes this primary drug effect. The widely divergent affinities of the sulfonamides might be attributed either to differences in acid strength or to specific influences of various rings upon the bond energy of the drug-albumin complex. By analogy to the fatty acids, and to the experiments described below, the latter explanation would appear more probable. The equal binding of sulfanilamide and sulfacetimide appears paradoxical since the former is little ionized and the latter completely ionized at physiological pH, for it is hardly possible that the negatively charged  $\text{SO}_2$  group plays no part in the primary combination (98). The facts can be interpreted to show that sulfacetimide actually has considerably greater affinity for albumin than does sulfanilamide, since it is bound to the same degree despite its much lower effective ionic concentration.

A searching comparative study of 41 substituted sulfadiazines has been reported by van Dyke and his associates (264). The substituents were alkyl, methoxy and ethoxy groups, simple alkyl ethers, and hydroxy or phenoxy radicals. Almost any substitution on carbon atom 4, 5 or 6 increased the binding to albumin and substitution in position 4 or 6 was more effective than in position 5. The affinities of the resulting compounds differed widely, from sulfadiazine itself ( $\beta = 32$ , at 10 mg %) to 4-methyl, 6-phenoxy sulfadiazine ( $\beta = 92$ ). The

increased binding of sulfamerazine (4-methyl) over the parent compound was confirmed, as well as the failure of a second methyl group in position 6 (sulfamethazine) to enhance the binding further. There was no obvious relation between affinity for albumin and either solubility or acid strength nor was it obvious what kind of substituent most enhanced the binding. However, the acid strengths of most of the compounds were not determined so that the degrees of ionization remained unknown. Consequently some of the data, based upon total concentrations, may be misleading.

The influence of positional factors is brought out with great clarity by Teresi and Luck (259), who determined  $K$  and  $n$  for a series of aromatic anions interacting with bovine albumin. Some molecular alterations were reflected in distinct differences in the maximum binding capacity ( $n$ ) of the albumin, but others produced a change in affinity ( $K$ ) without affecting the value of  $n$ . For example, 25 moles of phenylacetate could be maximally bound per mole of albumin ( $K = 23 \times 10^{-5}$ ) but the maximum binding capacity for phenoxyacetate was only 7 ( $K = 1.35 \times 10^{-5}$ ). The introduction of a single oxygen atom between the ionic group and the aromatic ring makes 18 previously available groups no longer accessible. At the same time the strength of binding to the remaining groups is significantly increased. Similar phenomena were noted with the nitrophenolates. When the nitro group was in the *m*- or *p*- position the value of  $n$  was 22 to 25, but in the *o*- position it was reduced to 6. That the ortho substituent actually abolishes the possibility of interaction with 16 to 19 otherwise available groups is made plain by the fact that a para substitution in the same molecule (2, 4-dinitrophenol) no longer has the usual effect, the value of  $n$  remaining fixed at 6. Nor does a third nitro-group (picrate) alter matters. On the other hand albumin complexes with di- and tri-nitro compounds have higher bond energies (greater affinities) than with the mono-substituted anions.

In a series of iodinated anions investigated by Smith and Smith (239), affinity for albumin decreased in the order hippuran > iopax > diodrast > neoiopax > skiodan. Unfortunately the sequence entails several simultaneous molecular changes. Between hippuran and neoiopax there is an increase from one to two iodine atoms, shift of a ketone group from a position near the carboxyl to the opposite end of the molecule, and finally (in neoiopax) a reduction in length of substituent carbon chain and appearance of a second carboxyl. Although it is not clear to which of these structural differences the progressive diminution in binding is to be attributed, it is of some interest that the order of affinity might have been predicted from the general principles elucidated in more complete investigations of other anions. Priodax, containing two aromatic rings, would probably be bound even more firmly than hippuran but its interaction with pure albumin has not yet been studied. A direct comparison between hippuran and hippurate would be particularly revealing with regard to the specific influence of iodination.

The interactions of dyes have been extensively studied, principally because it is so simple to determine their concentrations. Methylene blue was the first substance shown conclusively to interact with a soluble protein (Bechhold, 1907



(6)) As one might expect, both acidic and basic dyes combine with albumin only in their ionized forms (13). Indicators therefore only interact in one colored form, but some (*e g*, phenol red) dissociate doubly, carrying a charge in both forms. The great number of permutations commercially available in each dye group provides an unparalleled opportunity for studying the influence upon interaction affinity of molecular size, ring structure, character and position of ionic groups, various polar and non-polar substituents, and steric factors. But most interaction studies with dyes have been incidental to other problems, few systematic investigations having been directed toward the relation of affinity to structure among closely similar compounds. A few of the more interesting investigations are included in Table 1, and numerous other studies are indexed in Table 1a.

It has been suggested as a general rule that affinity is increased by large molecule size, particularly by aromatic rings which can contribute sizable van der Waal forces to the bond energy. The rings should be planar to facilitate close approximation to the protein surface, there should be no steric hindrance to approach, and polar groups should not be placed where their affinity for water can oppose the protein affinity of the whole molecule (138-140, 230).

These ideas emerged from the elegant studies by Klotz on several sulfonated azo dyes. *Orange I and II* contain a single sulfonate group on a phenyl ring in the para position to the azo link, and a hydroxyl group on the naphthyl ring. In orange I this hydroxyl is para to the azo link but in orange II it is in the ortho position, where an intramolecular hydrogen bond can be formed. As the affinity of orange II is appreciably greater than that of orange I, Klotz concludes that a polar substituent diminishes affinity for albumin unless its position permits reduction of its hydrophilic character (139). Azosulfathiazole consists of a sulfathiazole molecule joined through an azo link to a disulfonated naphthyl ring containing an ortho hydroxy residue. The curves obtained in saturation experiments indicate increasing difficulty of approach for successive molecules after albumin has bound the first few. This is attributed to electrostatic repulsion resulting from the presence of more than a single anionic residue. Nevertheless, this dye is bound about as firmly as *methyl orange*, which contains a single sulfonate group. As the latter contains two phenyl rings and no naphthyl, the data are interpreted to show that the larger molecular size and naphthyl ring of azosulfathiazole stabilize the dye-albumin bond despite the opposing effect of the multiple charges (138, 139). Other examples of the enhancing action of large aromatic rings on binding to albumin are found in a penicillin series recently investigated by Klotz (142). A bromophenylazo-hydroxybenzyl penicillin was bound more strongly than penicillin K, and a naphthylazo analogue showed even greater affinity. Likewise, in the experiments of Thumann and Rothschild (260),  $\alpha$ -naphthalene acetate was bound more strongly than indole-acetate. On the other hand, *amaranth*, which contains two azo-linked naphthyl rings, has three sulfonate groups, symmetrically placed on the outer edges of the molecule. Despite the favorable ring structure, this dye shows less affinity than azosulfathiazole and methyl orange (140). Klotz concludes that symmetrical charge

arrangements are, in general, detrimental to interaction with albumin, for the reasons stated above

Another example of the influence of symmetrical ionic groups is found in the studies by Rawson (201) on Evans blue and three related dyes. Evans blue and trypan blue are toluidine dyes containing two toluene rings azo-linked to two naphthyl nuclei, with two sulfonate groups on each of the latter. Niagarsky blue 6B and Niagara sky blue are dianisidine dyes, differing from the toluidine structure only in the replacement of methyl by methoxy in each benzenoid ring. The difference between the first and second member of each pair lies wholly in the position of the sulfonate groups on the naphthyl rings. In Evans blue and Niagara sky blue 6B these are in positions 2 and 4, along one edge of the molecule, while in the other two they are in positions 3 and 6. There is no striking difference between the binding of toluidines and dianisidines but both 2,4 sulfonates are bound very much more firmly than the more symmetrical 3,6 isomers. Gregersen and Gibson (107) had made the earlier observation that the absorption spectra of these dyes were also influenced by change in position of the sulfonate groups but not by the shift from methyl to methoxy in the phenyl rings.

That symmetrical *non-ionic* substitutions have the opposite effect of increasing affinity is shown by the behavior of a series of triphenylmethane dyes. Binding increased in the order basic fuchsin < malachite green < brilliant green < crystal violet, these dyes differing by the progressive substitution of dialkyl-amino groups in the para positions of all three rings (126).

Further pertinent information bearing on positional factors emerges from the recent work of Bueding (30, 31) showing that 1,2 naphthoquinones are bound more completely than 1,4 naphthoquinones. The introduction of an aliphatic side-chain in position 2 of a 1,4 naphthoquinone diminished the extent of interaction and further substitution of aliphatic groups in position 3 led to a greater reduction of affinity. That the 1-4 edge of the molecule is primarily involved in the interaction was clearly evidenced by the indifference of the binding to aliphatic substituents in the 5-8 positions. It might be predicted, however, that polar groups in the latter positions would diminish the extent of interaction.

The interactions of alkaloids and other organic cations have not been investigated with sufficient attention to the factors determining specificity. The semi-quantitative work of Beutner (14, 15) showed that pilocarpine was more firmly bound than other alkaloids studied. It has also been shown that a number of alkaloids combine (with gelatin) only on the alkaline side of the isoelectric point (190), suggesting combination with anionic protein groups. Interactions of alkaloids with pure albumin have not yet been systematically examined.

The cardiac glycosides are outstandingly important drugs whose binding to albumin is not obviously of an ionic character. Cholesterol and other steroids combine primarily with globulins. The bile salts, which contain additional hydroxyl groups and an aliphatic carboxyl residue in position 17, combine primarily with albumin (11, 49). The active principles of the cardiac glycosides closely resemble both cholesterol and the bile acids, sharing with the former its lack of a distinct ionizing group, and with the latter their content of more than a single

hydroxyl Like the bile salts they are bound by albumin and unlike other steroids they do not interact with globulins (85, 112, 215) The unsaturated lactone in position 17 is essential to the pharmacological activity but its specific influence on binding affinity is unknown

Most investigators agree that a particular glycoside and its aglycone are bound to the same degree (85, 112) If this is correct, it follows that the sugar residue is of no importance in interaction, and the same must be true of the hydroxyl in position 3, which is involved in the glycoside linkage (83, 89) However, few investigators have directed their experiments toward decisively settling this question Pairs of glycosides containing the same aglycone and different sugars should be studied (*e.g.*, digitoxin and diglanid A, k-strophanthin- $\beta$  and cymarín) as well as the corresponding aglycones themselves

The most complete comparative investigation of binding showed that affinity decreases in the order oleandrin > diglanid B > digitoxin > k- or g-strophanthin > "digitalin" > diglanid C > k-strophanthosid (112) As the various aglycones differ chiefly in the number and position of hydroxyl groups it would be interesting to correlate affinity for albumin with this single variable No quantitative data on the binding of pure aglycones are available, and the frequent use of impure mixtures has led to considerable confusion

If the difference in sugar residue can be ignored, the greater binding of oleandrin than of diglanid B must be caused by the difference in a single group Oleandrin differs from gtoxygenin only in acetylation of its hydroxyl in position 16, adjacent to the lactone ring (89) The marked reduction of affinity from diglanid B to diglanid C (containing the same sugars) may be caused by the shift of a hydroxyl from position 16 to the exposed position 12, on the "upper" edge of the molecule It may also, however, be connected with the unique steric arrangement ( $\alpha$ ) of the 3-hydroxyl of digoxigenin which forces the sugars of diglanid C into a different plane from those of diglanid B (89) Complete removal of the 16-hydroxyl of diglanid B, yielding digitoxin, does not lead to enhanced binding but to a slight diminution However, diglanid B contains a sugar not found in digitoxin

Several investigators have observed that k-strophanthin is bound poorly or not at all (85, 127, 159, 182, 215), but as the methods employed were biological (frog heart) a rapidly reversible interaction might not have been detected Employing *in vitro* technique, Haarman (112) found that k- and g-strophanthin were significantly bound but (as confirmed by Rothlin (215)) pure k-strophanthosid was not If, as seems probable, the aglycone strophanthidin has a very low order of affinity for albumin, it must be attributed to the aldehyde group in position 10 The poor binding of both strophanthidin and diglanid C would seem to show a deterrent effect of polar substituents along the "upper" edge of the phenanthrene nucleus, reminiscent of an analogous effect in the dye orange I (p 141)

Further quantitative investigations of the cardiac glycosides and aglycones should not only clarify the influence of the sugars but should also reveal whether the lactone represents a point of primary attachment to albumin, and whether

the albumin-aglycone bond is to be regarded as entirely non-ionic. If the latter is the case, variation of pH and consequent alteration of charges on the protein should not affect the extent of interaction. In this connection Hoekstra (127) claimed that the digitoxin-albumin combination dissociated above pH 8.3, a finding not confirmed by subsequent investigators (85, 86, 113).

*Substances that apparently do not interact with plasma proteins* For the reasons outlined in the discussion of methods, failure to obtain evidence of interaction may often be the result of inadequate technique, particularly when the possibility of binding is not explored at low enough drug concentration. An instructive example of this type of error is to be found in the work of Paget and Vittu (185), who sought to demonstrate the sulfanilamide-serum interaction by change in refractive index of a protein solution. Apparently because the method was not sensitive enough to small changes, concentrations of 250 to 500 mg % were employed. Of course, no interaction was detected because at these concentrations the *fraction bound* is entirely negligible so that the change in refractive index was essentially that to be expected from the total sulfanilamide concentration.

There is general agreement (11, 224) that sodium and potassium ions are not appreciably bound. From solubility data (183) it is to be concluded that nitrous oxide is not bound in plasma, and the same is true of ether if Haggard's (114) data are accepted over those of earlier workers (174).

Urea is generally regarded as unbound in the plasma. The electrophoresis studies of Bennhold (11) are offered in support of this view but they were carried out at concentrations over 100 mg %. On the other hand evidence in favor of interaction under physiological conditions has been obtained by three other investigators (153, 159, 214) while the denaturant action of urea on albumin at high concentration is well known (22, 24).

The question of a reversible interaction between glucose and plasma proteins must be left open. Bennhold (11) was unable to observe any migration of glucose with either albumin or globulin in an electric field, but de Haan (60) claimed that in bovine serum about 25 % of the glucose is not ultrafiltrable. Moreover, glucose compounds are found in the glycoprotein conjugates of fraction IV-6 (171, 252).

Streptomycin, like glucose, is highly polar, and interacts weakly if at all. Several investigators (142, 262) have been unable to observe binding by physical methods but antibacterial potency is unquestionably reduced in the presence of albumin (122, 142). This suggests that binding may be found only at concentrations too low to determine by non-biological methods. In explanation of these findings it has been proposed that the hydroxyls arrayed about the streptidine ring outweigh the expected electrostatic attraction of the charged guanidine groups for anionic centers on the albumin (59).

All but one of the porphyrins interact with albumin (3, 11, 50, 97, 164, 165, 210), while bilirubin (49, 165) and hematin (181) also form specific complexes with globulins. The coproporphyrins contain a single carboxyl group at the outer edge of each of the four nitrogenous rings, and these compounds migrate

with albumin. Addition of four more carboxyls leads to the uroporphyrin structure in which every reactive position on the periphery of the molecule is occupied by an ionic group. The uroporphyrins do not interact with either albumin or globulins (11, 97).

The probability that strophanthidin and its glycosides do not interact with albumin has been considered above. The failure to demonstrate any binding of thiamine has also been discussed. It cannot be accepted as proved that this vitamin is entirely unbound at normal plasma concentrations. It has been claimed that histamine and choline do not interact with plasma proteins (14), but while they obviously are not bound as extensively as some other alkaloids, the possibility of some interaction has not been adequately ruled out.

*The location of binding sites on the protein surface.* Attempts to localize interactions to specific reactive groups of plasma proteins were obviously out of the question until one of them had been completely purified and characterized with respect to content of amino-acids and their acidic and basic dissociation constants. A few bold thrusts at the problem have now been reported in connection with albumin interactions.

Klotz (140) has found that the combination of methyl orange and azosulfathiazole with albumin, as measured by spectral alteration, is constant as the pH is varied between 5 and 9 but complete dissociation occurs between pH 9 and 11.5. This corresponds very closely with the dissociation range of the  $\epsilon$ -amino groups of lysine, histidine giving up its protons at about pH 7, and arginine guanidinium groups remaining charged up to pH 12.6. It was therefore concluded that these dyes probably combine with the  $\epsilon$ -amino groups of lysine. It was not obvious why the arginine groups should have been unavailable since protamines, consisting largely of arginine, interact strongly with various sulfonates. On the other hand, these dye interactions yield a value of 22 for  $n$ , on the basis of which Klotz (138) had earlier concluded that the combination was with arginine, since the figure corresponded so closely to the total number of residues of this amino-acid. There are 59 lysine residues and if, as the pH variations now suggest, the dyes interact with  $\epsilon$ -amino groups, only half of them must be involved. This would raise the perplexing question why half the lysine amino groups should be inaccessible.

The experiments of Teresi and Luck (259) with aromatic carboxylic acids and nitrophenols led to values of  $n$  in two categories, some falling between 22 and 25, others between 6 and 9. The former corresponds, as stated above, with the number of arginine residues, but it is improbable that these anions would continue to interact up to pH 12.6 since the small change from pH 7.6 to 8.2 led to a diminished affinity in all those cases where the interacting compound itself was fully ionized. Values of  $n$  between 6 and 9 correspond roughly to the number of terminal  $\alpha$ -amino groups but it seems doubtful that these are specifically involved in the interactions.

From the standpoint of an approaching small molecule the individuality of the separate amino-acids is probably submerged in the totality of the surface pattern of the protein. Much as the doors and windows of a house cannot be

defined in terms of the characteristics of the component bricks, so may a specific binding site represent a new quality that is more than the sum of amino-acid residues. The characteristics of each such site may depend not only upon the properties of its ionic groups but also upon those of neighboring non-ionic residues, and upon steric hindrances to approaching molecules, and surface configurations affecting the strength of van der Waal forces. If there is any truth in such a concept the occasional correspondence between a value of  $n$  and the number of residues of a particular amino-acid must be regarded as fortuitous. The nature of the binding sites would then have to be elucidated by methods considerably more refined than have yet been employed.

**PHARMACOLOGICAL SIGNIFICANCE OF DRUG-PROTEIN INTERACTIONS** Most drugs, at therapeutic concentrations, interact with one or more of the plasma proteins. Before any pharmacological significance can be attributed to these interactions, three fundamentally different possibilities must be considered.

- (1) Drug bound to plasma protein is pharmacologically active
- (2) Only unbound drug is active but the drug-protein complex can penetrate to the sites of drug action, where special mechanisms transfer the drug molecule to receptor proteins
- (3) Only unbound drug is active or freely diffusible and the drug-protein complex is generally confined to the circulating plasma

The first possibility can be dismissed at once. Biological methods for the study of interactions owe their usefulness to the diminution of drug activity caused by interaction. Microbiological techniques permit direct proof that the potency of an antibacterial drug in the presence of protein is exactly that of its *unbound* fraction, which can be independently estimated by a non-biological method. The same proof is available for any drug whose potency is assayed against an isolated organ in the presence and absence of plasma protein.

The second possibility—that plasma proteins act as carriers for drugs—requires more serious consideration. Bennhold (11, 12) observed that numerous substances appeared to be completely and irreversibly bound at usual concentrations. At a loss to explain how these compounds could diffuse out of the vascular channels, he proposed a theory of "directed transport" according to which the carrier proteins themselves escape from the blood stream and enter tissue cells. There still remained the problem of intracellular liberation of bound drug, which could be resolved only by postulating special dissociation mechanisms in the various organs.

In support of this theory Bennhold cited experiments of von Jancsó (265), Schulten (227), and Plattner (193)<sup>4</sup>. These showed that under very limited conditions certain dyes and colloidal gold suspensions perfused through liver or kidney do not enter the cells of these organs *until* albumin is added to the perfusion fluid. One then observes prompt uptake by reticulo-endothelial cells in the liver, or excretion by the tubules of the kidney. So many anomalous results complicated these experiments that the investigators themselves were unwilling to draw any general conclusions. It is noteworthy that the results were obtained

<sup>4</sup> Incorrectly attributed to Höber and Titagew

with colloidal substances which are known to be disaggregated by albumin. Certainly the conclusion that albumin combines with the colloidal molecules and carries them over into the cells is not warranted.

Further support for the "directed transport" theory was drawn from the studies of de Haan (62, 63) on the phenomenon of phagocytosis, where there is good evidence that ingestion of particulate and colloidal matter is facilitated by protein. But there seems little justification for extending these observations to other cells and physiological membranes.

Hoekstra (127, 128) found that protection against the toxicity of digitoxin for the frog heart was afforded by rabbit serum but not by frog serum, while in the rabbit heart the efficacy of the two sera was reversed. It was concluded that the complex of digitoxin with homologous protein must enter the heart, a feat of which heterologous protein is incapable. These provoking findings have not been confirmed by other investigators.

While the relative impermeability of the glomerular membrane to protein seems well established (82, 202), certain capillaries must evidently allow passage to albumin molecules (96). The protein content of cervical lymph in dogs may be as high as 2.6% (217), most of which must have escaped from the vascular channels. Gregersen and Rawson (108) attributed the disappearance of Evans blue from the blood stream to escape of albumin since the dye is almost completely albumin-bound, and this disappearance is exceedingly slow. The well-devised experiment of Pfaff and Herold (191) also indicates a negligible escape of albumin. When a mixture of green and blue dyes was injected intravenously in a rabbit and the mesentery observed microscopically, the green dye, which was completely bound to plasma protein, remained well confined within the vascular channels, but the less completely bound blue dye promptly diffused into the tissue spaces. Only when severe inflammation was artificially induced did the green dye appear beyond the capillary walls, presumably still attached to escaping albumin molecules. However, the ultimate mode of disappearance of the green dye from the blood stream was not investigated.

As the chain of logic that forced Bennhold to the theory under consideration originated in the apparent irreversibility of a number of interactions, the nature of the evidence for such irreversibility will be reviewed briefly.

If it is sometimes difficult to demonstrate reversibility, it is almost impossible to prove irreversibility. Most drug-protein complexes, as Table 1 shows, dissociate readily upon simple dilution or dialysis, because an appreciable concentration of unbound drug is in equilibrium with them. When dissociation constants are small and drug concentrations low, apparently stoichiometric complexes are formed. These, too, should dissociate readily whenever the equilibrium is disturbed by rapid removal of unbound drug, no matter how low its concentration. However, such methods as ultrafiltration and electrophoresis that do not markedly alter an equilibrium would not permit the detection of unbound drug in such cases, and irreversibility could neither be proved nor disproved. Therefore Bennhold's frequent observation that *all* of a substance migrated electrophoretically with a protein fraction is not a valid basis for his conclusions about irreversibility.

Even prolonged dialysis does not suffice to rule out simple reversibility because

the rate of diffusion out of the bag is proportional to the concentration gradient across the membrane. If extremely little unbound drug were present none might be detected in the dialysate in any reasonable period of time. Heymann and Fieser (124), finding that the hydroxy-naphthoquinone M-1971 (at 116 mg %) did not dialyze out of albumin, placed the bag in another albumin solution. This should have trapped any drug diffusing out, preventing back-diffusion. As none appeared in the dialysate under these conditions it is fair to conclude that very little unbound drug was present. However, even this novel technique does not overcome the objections stated above, and irreversibility cannot be regarded as proved. In the capillaries, or in direct contact with microorganisms, circulating plasma is exposed to so phenomenal a surface area for diffusion that conclusions about irreversibility based on dialysis might be entirely invalid.

There would appear to be at least two fairly rigid tests of reversibility. The drug-protein complex might be greatly diluted, followed by separation of the protein (e.g., by ultrafiltration) and determination of drug concentration in the water phase after evaporation to the original volume, or by adsorption techniques. Alternatively an adsorbent of high affinity for the drug might be introduced into the original solution containing the complex. Kimmig and Weselmann (134) were thus able to show that a highly bound sulfonamide that could not be detected in an ultrafiltrate of plasma was easily removed by charcoal. In either technique there must be assurance that the integrity of the protein is not destroyed.

The foregoing remarks are not intended to detract from the fundamental importance of Bennhold's contributions to this field but only to indicate, with Rothlin (216), that a "vehicle function" of plasma protein cannot be accepted in the teleological sense of a specially-designed mechanism for directed transport of various substances, including drugs. The means whereby the several globulin conjugates are split to make the molecules they carry available for metabolic processes are not understood. But the behavior of drug-protein complexes is quite compatible with mass law principles and the main effects of interaction upon the fate of drugs in the body can be analyzed without recourse to special theories of transport or unique mechanisms of dissociation.

The primary effect of interaction, then, as suggested in the third possibility proposed at the beginning of this section, is to confine drug molecules within the blood stream in an inactive form, and thereby hinder their access to the sites of drug action, excretion and metabolism. This concept is illustrated schematically in Figure 9. It was first formulated (in extension of Ehrlich's ideas) by Storm van Leeuwen (248, 249) who proposed that drugs combine not only with so-called *dominant receptors* responsible for primary pharmacological action, but also with numerous *secondary receptors* in the body tissues. The effect and fate of drugs *in vivo* was considered to depend upon the *distribution* of drug molecules between the dominant and secondary receptors. In the plasma, the secondary receptors were identified as the plasma proteins.

*Drug excretion and diffusion.* Protein binding played a pivotal role in the development of current concepts of renal physiology, and the first proof of tu-



tubular secretion (Marshall and Vickers (161)) rested upon the large discrepancy between the rapid rate of excretion of phenol red and its poor ultrafiltrability from plasma. Subsequent studies led to the consistent result that the fraction filtered at the glomeruli corresponds to that ultrafiltered *in vitro* (162, 192, 229). That essentially all the dye is removed in a single passage of blood through the kidney (162, 235) despite the fact that 80% is in protein combination implies that protein-bound molecules are fully available for tubular secretion, and also that such protein complexes can dissociate with extraordinary rapidity<sup>5</sup>

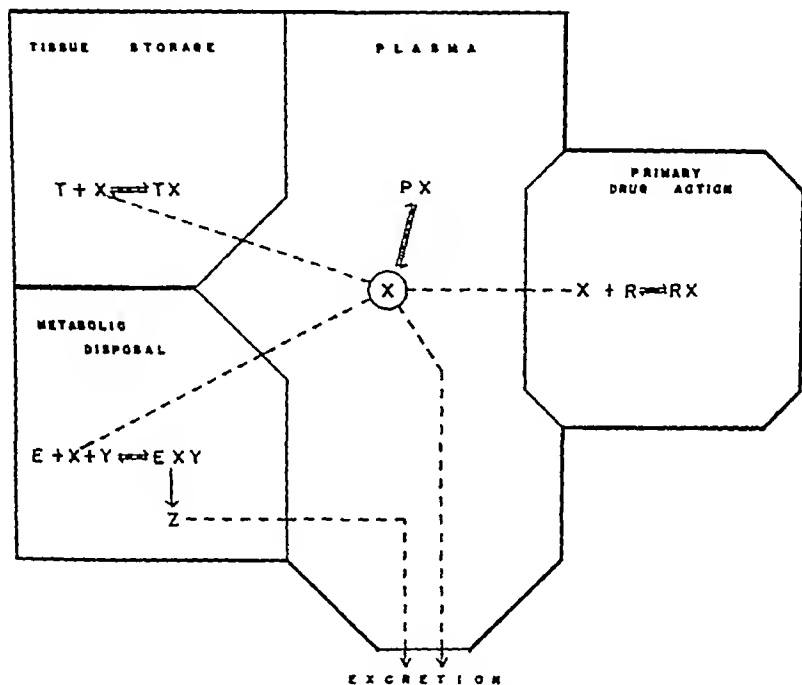


FIG 9 POSTULATED INFLUENCES OF VARIOUS REVERSIBLE INTERACTIONS, INCLUDING THOSE WITH PLASMA PROTEINS, UPON PRIMARY DRUG ACTION

The divergent effects of interaction upon glomerular and tubular excretion amply confirmed with numerous substances, are entirely reasonable. As no dilution is entailed, the filtration of about one-fifth of the plasma water at the glomeruli does not disturb reversible equilibria. Only when the plasma volume is reconstituted in the lower nephron would bound drug be expected to dissociate. But the rapid removal and excretion of unbound drug by the tubule cells would of necessity lead to dissociation of a drug-protein complex.

The behavior of phenol red at the tubules is duplicated by penicillin G which

<sup>5</sup> Whether all drug-protein complexes are capable of dissociating as rapidly is still unknown but would seem unlikely. Study of the kinetic aspects of both combination and dissociation should yield information of prime theoretical and practical importance.

is more than half bound to plasma protein (74), and by diodrast and hippuran (235) which are less completely bound (239). That there is but a single mechanism of tubular secretion is evidenced by the competition of all these substances for excretion (157, 235) and it is of some interest that they also compete for attachment to plasma albumin (239).

Obviously no valid statement can be made about the mechanism of renal excretion of any substance without full knowledge of its fractional binding at the concentration employed. It is incorrect to assume that a drug is filtered and resorbed, simply filtered, or filtered and secreted, on the basis of clearance with respect to that of inulin. Clearance must first be calculated on the basis of *ultrafiltrable* plasma concentration. Fortunately, neither inulin nor creatinine appear to be bound appreciably at concentrations usual to clearance studies.

The sulfonamides, because of their low clearances, had been generally assumed to leave the plasma by glomerular filtration alone, but Lundquist (157) presents sound evidence that the filtrable fractions of sulfathiazole and sulfamethylthiadiazole are not large enough to account for the clearances observed. The conclusion that these drugs are partly excreted by the tubules is confirmed by the effects of diodrast and phlorhizin, both of which reduce the sulfonamide clearance by about two-thirds, to the filtration level. Caronamide, which inhibits the tubular excretion of other substances, was also initially thought to be cleared solely by glomerular filtration but allowance for protein binding again permits the conclusion that some, at least, is excreted by the tubules (77).

The mechanism of hepatic excretion is poorly understood but evidently, as at the renal tubules, both unbound and bound drug can be removed from the plasma. Thus bromsulfalein, despite its high fractional binding, is quantitatively excreted in the usual liver function test (211). Brauer (26) observed, however, that in perfused or sliced rat livers, the *rate* of uptake of the dye was much reduced by albumin.

Diffusion of drugs and other substances into the cerebrospinal fluid has been the subject of numerous investigations that are outside the scope of this review. With respect to most diffusible molecules cerebrospinal fluid is generally regarded as an ultrafiltrate of plasma (60, 61, 64). Davis' (53-55, 57) proof that this is true of the sulfonamides and the practical therapeutic implications of his findings are too well known to require restatement here. The diffusion of a number of dyes from the blood into various body fluids is unquestionably hindered by protein binding (61, 62, 99, 108, 124, 191, 231).

Plasma urate presents a curiously anomalous behavior, for despite an ultrafiltrable fraction of 80%, it appears in cerebrospinal fluid to only 6% of its total plasma concentration, and its renal clearance is correspondingly low (276). It is postulated by Wolfson and his associates (277, 278) that about 70% of the total exists in some unique non-diffusible form, probably polymeric in nature, and some experiments on normal and azotemic chickens (151) lend support to this hypothesis. If such a polymer exists the cerebrospinal fluid urate would presumably be in equilibrium with the monodisperse plasma urate. It is not clear why the supposed polymer should pass so freely through an ultrafiltration membrane.

*Drug persistence* Some drugs are selectively concentrated and stored in certain organs, yet the process is obviously reversible since they all slowly disappear from the body. One wonders whether this binding to fixed tissues is not wholly compatible with the principles governing reversible binding in the plasma. The effective intracellular protein concentration in various organs (for example, the liver) is obviously considerably higher than would appear from a calculation of gross protein content. If the affinity of these proteins for a drug is very great, "complete" binding would be expected to result, and the total amount that could be thus bound and stored would be roughly determined by the mass of the organ. An extremely minute circulating plasma level might then be in equilibrium with large amounts of drug so stored. After a large single dose of such a drug one would expect a rapid disappearance from the plasma and excretion into the urine, fractional binding being low while drug concentration is still high. But the excretion rate should soon become negligible while large amounts of drug remain in the body to be released very slowly from tight combination with fixed tissue and plasma proteins. This description will be recognized as typical of the behavior of such substances as emetine, the organic arsenicals and antimonials, quinacrine, and suramin.

The case of suramin is a particularly remarkable one. Although there is no selective storage in any fixed tissue (20), the drug confers protection against trypanosomiasis for weeks to months after a single injection (167), and its actual presence in the plasma for this period has been demonstrated (241). That it is firmly bound to plasma protein is well established but it is not entirely clear whether albumin or a globulin is primarily responsible (66, 143, 168). Spinks (241) has shown that interaction depends upon the enormous molecular size, aromatic polypeptide structure, and terminal sulfonate groups, and that persistence, in a series of related compounds, can be roughly correlated with affinity for plasma proteins. It has been suggested that the colloidal nature of the molecule makes it ineligible for renal excretion (59) but such excretion does occur during the first few days after injection (20, 241).

The iodinated aromatic anion, priodax, also persists in the plasma for months, apparently tightly combined with protein (205, 206). To what extent differences in the persistence of other drugs are also to be explained on the basis of interaction with plasma or tissue proteins cannot be decided from the meager data at hand.

*Chemotherapy* The fact that suramin is therapeutically active despite its nearly complete binding might seem paradoxical. In Davis' (59) opinion the contrasting effectiveness of suramin and ineffectiveness of penicillin K in plasma is to be explained on the basis of relative potency. Quinacrine, for example, and related amino-acridines, are bound as much as 98% at therapeutic levels, the unbound 2% evidently sufficing to inhibit plasmodial growth (254). The diamidines are so highly bound in plasma that their antibacterial action is abolished (92), but they remain effective against trypanosomes. The therapeutic action of naphthoquinones against plasmodia (124, 238) and schistosomae (30, 31) is markedly reduced by plasma protein. The impressive inhibition of growth of the tubercle bacillus by oleic acid is nullified by traces of albumin (56, 58).

But penicillin G and sulfadiazine are effective antibacterials despite fractional binding of more than 50%

The variant and unpredictable influence of plasma protein binding upon the behavior of different chemotherapeutics toward a variety of organisms emphasizes the wisdom of avoiding generalizations in this field. For example, the simple statement that protein binding antagonizes the antibacterial action of penicillin K obscures a possibly important therapeutic advantage. This drug is over 90% bound at concentrations considered adequate for other penicillins (261). If the fractional bindings were not much smaller at very much higher concentrations (cf. Figure 7) a significant therapeutic advantage could be gained. The unbound 10% would exert the desired antibacterial effect and the bound 90% would dissociate as excretion proceeded, to "buffer" the unbound penicillin level. The plasma proteins would provide a flexible reservoir of penicillin serving to damp otherwise rapid fluctuations of level. It may be that if an effective *unbound* concentration can be maintained, the capacity for protein interaction is to be desired in a chemotherapeutic agent.

*Displacement of bound drug.* A surprisingly large number of casual observations have been reported showing that substances interacting with plasma proteins can be partially or completely liberated by introduction of other substances into the system. The reports are summarized in the eighth column of Table I.

In some cases there is obvious competition for binding sites, as in the reduction of anion binding by alkali and of cation binding by acid (178, 242), or, conversely, the displacement of titration curves when the character of the anion is varied (243). The depression of the binding of one anion by another has also frequently been observed and may be of some practical importance in the use of buffers for interaction studies. Phosphate, for example, interacts with albumin and may displace other anions (2). Barbitol (Veronal) significantly depresses the binding of phenol red (208). A number of carboxylic acids promote dissociation of complexes between anionic dyes and albumin (140a). Hippuran and diodrast displace phenol red from its albumin combination (239).

There are other instances of displacement which can reasonably be attributed to competition between compounds that are known to compete for physiological receptors or that are structurally similar. Atropine antagonizes the binding of pilocarpine (14). Bile salts and saponin were reported to split the digitoxin-albumin complex (127) but neither finding has been confirmed. Carbamic ester inhibitors are displaced by acetylcholine from their combination with the plasma cholinesterase (102) and flavoenzyme inhibitors are displaced by the enzyme prosthetic groups (121).

There remain a number of curious cases where the relation between the antagonistic substances is by no means evident. The displacement of pilocarpine was effected not only by atropine, as stated above, but also by benzene, xylol, several phenols and guaiacol (which itself interacted with serum) (14, 249). A series of aliphatic alcohols and their esters had the same action, hexyl alcohol proving most efficient (16). (Cf. the optimal stabilizing effect of  $C_7$  carboxylic acid (23).)

Bile salts were reported to liberate rose bengal, bromsulfalein and bilirubin from plasma combination (212, 213) but this could not be confirmed, and glycocholate has since proven ineffective as a displacing agent in several cases (85, 124). Heparin replaces cephalin in the combination with thromboplastic lipoprotein (38, 39). The tightly bound naphthoquinone M-1971, that could not be liberated by dialysis, readily dissociated from albumin upon addition of isoamyl alcohol (124).

Finally, ether, at anesthetic concentrations, was reported by Storm van Leeuwen (247) to dissociate the serum protein complexes of a number of alkaloids. Dale (52) showed that the sensitivity of cats to histamine is increased ten-fold by etherization, an observation that might be explained by displacement of histamine from combination in the plasma. However, there is still no good evidence that such a combination exists. It has been reported recently that etherization and dial-urethane both increase the passage of sulfathiazole into the cerebrospinal fluid. The investigators attributed the finding to vascular dilatation but noted that despite equivalent vascular changes chloroform does not duplicate the effect (257). The possibility of dissociation of the highly bound sulfonamide from albumin under the influence of ether was not considered.

These numerous observations, not yet falling into any intelligible pattern, pose stimulating questions for pharmacological study. There are obviously occasions, in the normal or pathological state, when the concentration of a drug may be sufficient to displace other drugs or normal plasma constituents from their protein combinations. If the fractional binding of such a displaced substance were initially great, the effect of the displacement could be a sudden and substantial increase in the unbound plasma concentration. It is not difficult to imagine the possible dangers such episodes might present, but whether they really occur, under what conditions, and with which competing drugs, is entirely unknown.

*Species and other differences in binding capacity* That the serum of different species varies considerably in the capacity to protect against drug effects has been appreciated for many years. These differences have frequently been observed with drugs known to be bound exclusively by albumin, so it may be inferred that chemical reactivity of the respective albumins is the principal variant. The superior binding properties of rabbit serum toward various drugs has been almost universally noted. Beutner (14) found the relative effectiveness of several sera in binding pilocarpine to be rabbit > goose > sheep > human > horse > bovine, while pig serum was as ineffective as egg albumin. Storm van Leeuwen (245) reported the sequence rabbit > cat = bovine > dog for the same compound. In both studies the order of affinity was the same for other alkaloids as for pilocarpine. In the binding of cardiac glycosides Farah (85) found the order rabbit > dog > human > frog. Kocian (143) studied the stabilizing effect of suramin against protein coagulation and reported the order carp > duck > human > horse > pig = bovine = rabbit > goose > chicken. Recently Wendel (288) and also Heymann and Fieser (124) showed that naphthoquinone antimalarials are bound to a very different extent by duck and human plasma. Moreover,

But penicillin G and sulfadiazine are effective antibacterials despite fractional binding of more than 50%

The variant and unpredictable influence of plasma protein binding upon the behavior of different chemotherapeutics toward a variety of organisms emphasizes the wisdom of avoiding generalizations in this field. For example, the simple statement that protein binding antagonizes the antibacterial action of penicillin K obscures a possibly important therapeutic advantage. This drug is over 90% bound at concentrations considered adequate for other penicillins (261). If the fractional bindings were not much smaller at very much higher concentrations (cf. Figure 7) a significant therapeutic advantage could be gained. The unbound 10% would exert the desired antibacterial effect and the bound 90% would dissociate as excretion proceeded, to "buffer" the unbound penicillin level. The plasma proteins would provide a flexible reservoir of penicillin serving to damp otherwise rapid fluctuations of level. It may be that if an effective *unbound* concentration can be maintained, the capacity for protein interaction is to be desired in a chemotherapeutic agent.

*Displacement of bound drug.* A surprisingly large number of casual observations have been reported showing that substances interacting with plasma proteins can be partially or completely liberated by introduction of other substances into the system. The reports are summarized in the eighth column of Table 1.

In some cases there is obvious competition for binding sites, as in the reduction of anion binding by alkali and of cation binding by acid (178, 242), or, conversely, the displacement of titration curves when the character of the anion is varied (243). The depression of the binding of one anion by another has also frequently been observed and may be of some practical importance in the use of buffers for interaction studies. Phosphate, for example, interacts with albumin and may displace other anions (2). Barbital (Veronal) significantly depresses the binding of phenol red (208). A number of carboxylic acids promote dissociation of complexes between anionic dyes and albumin (140a). Hippuran and diodrast displace phenol red from its albumin combination (239).

There are other instances of displacement which can reasonably be attributed to competition between compounds that are known to compete for physiological receptors or that are structurally similar. Atropine antagonizes the binding of pilocarpine (14). Bile salts and saponin were reported to split the digitoxin-albumin complex (127) but neither finding has been confirmed. Carbamic ester inhibitors are displaced by acetylcholine from their combination with the plasma cholinesterase (102) and flavoenzyme inhibitors are displaced by the enzyme prosthetic groups (121).

There remain a number of curious cases where the relation between the antagonistic substances is by no means evident. The displacement of pilocarpine was effected not only by atropine, as stated above, but also by benzene, xylol, several phenols and guaiacol (which itself interacted with serum) (14, 249). A series of aliphatic alcohols and their esters had the same action, hexyl alcohol proving most efficient (16). (Cf. the optimal stabilizing effect of  $C_7$  carboxylic acid (23).)

The antibody globulins obtained by these techniques can be characterized as follows. They react with the original antigen but the reaction can be blocked by the specific haptene employed, or by closely related compounds—i.e., the drug used for sensitization interacts specifically with the antibody. In the intact animal, or in isolated organs, the anaphylactic response can be elicited only once, if first blocked by the haptene drug it cannot be elicited at all. The extent of interaction between antibody and compounds closely related to the haptene drug may vary considerably. Gerber and Gross (95) showed that while cross-reactions occurred between sulfanilamide, sulfacetimide, and sulfanilic acid and their respective antisera, the specificity did not extend to sulfathiazole, para-amino-benzoic acid, or 2,4-nitroaminophenol.

Although the obvious practical aim in studies of this type would be to obtain antibodies capable of neutralizing certain drug effects *in vivo*, few very successful outcomes in this direction have been reported. Clutton and co-workers (43) presented evidence that antisera against thyroxyl-thyroglobulin protected rabbits against exogenous thyroglobulin or thyroxin, but no alteration of the normal basal metabolic rate was observed. Butler and his associates (34) found that antisera against aspirin-protein complexes protected rats dramatically against the antipyretic action of aspirin when artificial fever was induced. Fell and his collaborators (87) observed slight protection against the anaphylactic action of ovalbumin in guinea-pigs and rabbits by an anti-histamine immune serum. Singer (232) presented data purporting to show that the  $LD_{50}$  for mice injected with certain arsenicals was appreciably altered by an anti-arsenical immune serum. Hooker and Boyd (129) calculated the probable amount of antibody globulin produced in typical experiments of this kind and showed the improbability of a titer high enough to neutralize any significant proportion of injected drug, unless the drug were so highly potent that the therapeutic dose contained relatively few molecules.

It is difficult to understand how, if so drastic a coupling procedure as diazotization were required for the production of an antigen, such substances could ever arise *in vivo*, as they must, if drug allergy is to be explained on this basis. It is therefore highly significant that in a few instances antigenic substances have been produced without diazotization. Clutton, Harington and Yuill (42) starting from the premise that both tyrosine and carbohydrate play significant roles in normal antigenicity, linked glucose to tyrosine and the latter through a peptide bond to protein. Potent antisera to this conjugate were produced by rabbits when either horse albumin or globulin was used as carrier. A similar peptide-linked antigen was successfully produced from the isocyanate of histamine, which was presumed to react with the lysine  $\epsilon$ -amino groups of horse globulin (209).

While the linkages between haptene and protein seem more natural in these compounds than in the azoproteins, they are still far from the reversible bonds typical of drug-albumin complexes. A closer approach to the latter is found in the observation that the Wassermann or Forssman haptens become antigenic on simple contact with *heterologous* serum, no drastic treatment being required

the relative binding power of the two sera was different for each compound tested. Most drugs are bound to about the same extent by human, bovine and horse plasma, a fortunate coincidence since these are the common sources of albumin for experimental work. In general, however, the complete unpredictability of species differences and the variation of relative binding power with different drugs dictate caution in transferring interaction data from one species to another.

Differences in the binding capacity of human plasma have been meagerly examined. Farah (85) reported that in the binding of digitoxin by the serum of normal subjects there were distinct differences that proved, upon repeated test, to be characteristic of each subject.

Storm van Leeuwen (250, 251) claimed to have shown that the sera of asthmatics hypersensitive to aspirin had a diminished binding power toward the drug as compared with normals and other asthmatics. The data, however, do not show impressive differences.

The plasma proteins undergo profound alterations that disrupt the normal electrophoretic pattern, in a number of conditions, including even so acute an episode as lobar pneumonia (111a, 279). It is well known that the plasma proteins are grossly disordered in such states as nephrosis, cirrhosis, myeloma, the collagen diseases, and various parasitic infestations. In few of these has the binding capacity of the plasma proteins been studied.

The abnormal loss of congo red from plasma in patients with amyloid disease was regularly observed by Bennhold (11). In nephrotics without amyloid the binding capacity of serum toward the dye was also substantially below normal. It was subsequently shown that nephrotic serum has a depressed binding power toward many other substances and that this depression is disproportionate to the decrease in plasma albumin.

The possibility that impairment of the binding capacity of plasma proteins may be a significant factor in many diseases or may play a part in unusual sensitivity or resistance to drugs has not yet been systematically investigated.

*Drug allergy* The vexing question of drug allergy appears to be closely connected with protein interactions in general. Following Landsteiner's method of producing antigens by coupling foreign substances to proteins through diazo linkages, a number of investigators have succeeded in producing artificial drug antigens and obtaining specific antibody responses from experimental animals. Over twenty years ago Mayer and Alexander (170a) did this successfully with atoxyl and since then the experiment has been repeated in standard form with aminopyrine and related compounds (176, 177, 218), strychnine (129), epinephrine (269, 270), aspirin (34), histamine (87, 209), organic arsenicals (232), tyrosine (42), thyroxine (43), sulfonamides (95), and probably others. The drug, coupled firmly to protein, is injected over a period of several weeks into a test animal. The resulting antiserum can then be titrated by a precipitin reaction against the original antigen, or the anaphylactic response of the whole immunized animal or of its isolated organs can be elicited. The protein employed as antigen need not be heterologous. In some cases only albumins were suitable as antigens, purified globulins giving wholly negative results.



The antibody globulins obtained by these techniques can be characterized as follows. They react with the original antigen but the reaction can be blocked by the specific haptene employed, or by closely related compounds—i.e., the drug used for sensitization interacts specifically with the antibody. In the intact animal, or in isolated organs, the anaphylactic response can be elicited only once, if first blocked by the haptene drug it cannot be elicited at all. The extent of interaction between antibody and compounds closely related to the haptene drug may vary considerably. Gerber and Gross (95) showed that while cross-reactions occurred between sulfanilamide, sulfacetimide, and sulfanilic acid and their respective antisera, the specificity did not extend to sulfathiazole, para-amino-benzoic acid, or 2,4-nitroaminophenol.

Although the obvious practical aim in studies of this type would be to obtain antibodies capable of neutralizing certain drug effects *in vivo*, few very successful outcomes in this direction have been reported. Clutton and co-workers (43) presented evidence that antisera against thyroxyl-thyroglobulin protected rabbits against exogenous thyroglobulin or thyroxin, but no alteration of the normal basal metabolic rate was observed. Butler and his associates (34) found that antisera against aspirin-protein complexes protected rats dramatically against the antipyretic action of aspirin when artificial fever was induced. Fell and his collaborators (87) observed slight protection against the anaphylactic action of ovalbumin in guinea-pigs and rabbits by an anti-histamine immune serum. Singer (232) presented data purporting to show that the  $LD_{50}$  for mice injected with certain arsenicals was appreciably altered by an anti-arsenical immune serum. Hooker and Boyd (129) calculated the probable amount of antibody globulin produced in typical experiments of this kind and showed the improbability of a titer high enough to neutralize any significant proportion of injected drug, unless the drug were so highly potent that the therapeutic dose contained relatively few molecules.

It is difficult to understand how, if so drastic a coupling procedure as diazotization were required for the production of an antigen, such substances could ever arise *in vivo*, as they must, if drug allergy is to be explained on this basis. It is therefore highly significant that in a few instances antigenic substances have been produced without diazotization. Clutton, Harington and Yuill (42) starting from the premise that both tyrosine and carbohydrate play significant roles in normal antigenicity, linked glucose to tyrosine and the latter through a peptide bond to protein. Potent antisera to this conjugate were produced by rabbits when either horse albumin or globulin was used as carrier. A similar peptide-linked antigen was successfully produced from the isocyanate of histamine, which was presumed to react with the lysine  $\epsilon$ -amino groups of horse globulin (209).

While the linkages between haptene and protein seem more natural in these compounds than in the azoproteins, they are still far from the reversible bonds typical of drug-albumin complexes. A closer approach to the latter is found in the observation that the Wassermann or Forssman haptenes become antigenic on simple contact with *heterologous* serum, no drastic treatment being required

(267) The only recorded observations of similar spontaneous development of antigenicity with *homologous* serum, are those of Samson and Götz (218). Finding that aminopyrine at 0.4% in horse serum yielded no unbound drug in an ultrafiltrate, these investigators proceeded to sensitize guinea-pigs in the following simple way. Fresh blood was drawn from the animals and allowed to stand with aminopyrine for thirty minutes. The mixture was then injected intraperitoneally, the process being repeated at intervals of 5 to 10 days. At the end of this time an allergic response was obtained upon injecting a similar mixture (containing less drug) intravenously. Adequate controls ruled out non-specific factors but the phenomenon was peculiar in that the pure drug would elicit an allergic response if injected after sensitization. Moreover, pure aminopyrine in aqueous solution (without blood) could be used for sensitizing, but then a response was no longer elicited by the drug itself but only by the drug-blood mixture. No attempt was made to demonstrate antibody in the circulating plasma, and the experiments are open to criticism in other respects, but the observations conform so closely to the spontaneous development of clinical drug allergy that they might be profitably re-examined.

The fundamental question, of course, is whether the common interactions of drugs with proteins in the plasma are capable of giving rise to antigenicity, and if so, under what conditions. There is no particular evidence to suggest a parallelism between affinity for plasma proteins and allergic potentiality but this does not bear directly upon the question. Obviously interaction of a drug with plasma protein is not a *sufficient* condition for allergy, or *all* penicillin-treated patients would become sensitive to the drug. Whether interaction of the kind discussed in this review is a *necessary* condition remains to be proved.

\*     \*     \*

I would emphasize again, in conclusion, what is probably the most fundamental aspect of the interactions of drugs and plasma proteins. These diverse combinations must be regarded as model systems for elucidating the nature of the primary interaction of each drug with its protein receptor in the body. In isolated instances the affinity for plasma protein appears to parallel the primary potency of a drug. Davis and Wood (53) have shown that this is true in the sulfonamide series. Another parallelism has been indicated in the competition of certain substances for tubular excretion and for binding to albumin. But it is not generally the case. Even among the sulfonamides acetylation abolishes antibacterial action without affecting protein binding. Penicillin K and X, in the absence of albumin, are both more potent antibacterials than G, although the order of affinity for albumin is  $K > G > X$ . In the barbiturate series binding cannot be correlated with either therapeutic potency or susceptibility to metabolic destruction in the body.

Simple parallelisms are not to be expected. Only as we elucidate the nature of the forces that are responsible for interaction specificity, and begin to gain insight into the precise nature of the protein surface, shall we also be furthering our

understanding of the remarkable capacity for specialized protein interactions that distinguishes the potent drug molecule from its biologically indifferent kin

Many colleagues in this department and in the Department of Physical Chemistry unwittingly assisted in the preparation of this review by their willingness to engage in frequent discussions, and I thank them collectively. I am especially indebted, for their interest and assistance, to Drs. Bernard D. Davis, John T. Edsall, Otto Kraymer, George Scatchard, and Douglas M. Surgenor, and to Dr. Edwin J. Cohn, who first interested me in the interaction problem, and made protein fractions available for experimental studies. The cooperation of Mrs. Susan Jackson, Mr. Richard O. Bicks, and Mr. William Rutter is also acknowledged.

## REFERENCES

1. ANDERSEN, A. H. On fixation of sulfathiazole, sulfapyridine, sulfanilamide, and para aminobenzoic acid by plasma. *Acta Pharm. et Toxicol.*, 1: 141-148, 1945.
2. BALLOU, G. A., BOYER, P. D., AND LUCK, J. M. The electrophoretic mobility of human serum albumin as affected by lower fatty acid salts. *J. Biol. Chem.*, 159: 111-116, 1945.
3. BARAC, G., AND ROSEMAN, R. Faits expérimentaux sur la stabilisation anti-oxygénée de la bilirubine par les protéines plasmatiques et hypothèse sur la nature de la cénase albumino-bilirubinique du plasma. *J. Wash. Acad. Sci.*, 36: 295-301, 1946.
4. BASSETT, A. M., COONS, A. H., AND SALTER, W. T. Protein bound iodine in blood. V. Naturally occurring iodine fractions and their chemical behavior. *Am. J. M. Sc.*, 202: 516-527, 1941.
5. BAUER, H., AND STRAUSS, E. Über die Bindung komplexer Wismutsalze im Serum. *Ztschr. f. physiol. Chem.*, 149: 19-29, 1925.
6. BECHHOLD, H. Ultrafiltration. *Biochem. Ztschr.*, 6: 379-403, 1907.
7. BECHHOLD, H. Zur innern Antiseptik. *Ztschr. f. physiol. Chem.*, 52: 177-180, 1907.
8. BECHHOLD, H. Kolloidstudien mit der Filtrationsmethode. *Ztschr. physik. Chem.*, 60: 257-318, 1907.
9. BENDIEN, W. M., AND SVAFFER, I. Untersuchungen über die Bindung der Kolloide des Serums mit Hilfe von Ultrafiltern erhöhter Durchlässigkeit. *Biochem. Ztschr.*, 260: 105-114, 1933.
10. BENDIEN, W. M., AND SVAFFER, I. Weitere Untersuchungen über die Bindung der Serumkolloide mittels für Eiweiss durchlässiger Ultrafilter. *Biochem. Ztschr.*, 261: 1-14, 1933.
11. BENNHOLD, H. Die Vehikelfunktion der Blutweisskörper. Chapter in *Die Eiweisskörper des Blutplasmas*, by H. Bunnhold, E. Kylin and S. Rusznyak. Steinkopf, Leipzig, 1938.
12. BENNHOLD, H. Ist das Blutplasma ein strömendes Eiweissdepot oder ein Transportorgan? *Deutsche med. Wchnschr.*, 72: 401-404, 1947.
13. BETHE, A. Der Einfluss der H-Ionenkonzentration auf die Permeabilität toter Membranen, auf die Adsorption an Eiweisslösungen und auf den Stoffaustausch der Zellen und Gewebe. *Biochem. Ztschr.*, 127: 18-33, 1922.
14. BEUTNER, R. The binding power of serum for drugs tested by a new in vitro method. *J. Pharmacol. and Exper. Therap.*, 25: 365-380, 1925.
15. BEUTNER, R. The reaction between serum and alkaloids. *J. Pharmacol. and Exper. Therap.*, 29: 95-103, 1926.
16. BEUTNER, R., AND HYOEN, E. The binding power of serum for alkaloids and the inhibition of this effect by homologous alcohols. A contribution to the theory of narcosis. *J. Pharmacol. and Exper. Therap.*, 35: 25-30, 1929.
17. BIGGER, J. W. Inactivation of penicillin by serum. *Lancet*, 2: 400-403, 1944.
18. BJERRING, T., AND ØLLGAARD, E. Studies in sulphate clearance. *Acta med. Scandinav.*, 102: 55-78, 1939.
19. BOETTGER, E. G. The adsorption of estrin by the plasma proteins. *J. Cell and Comp. Physiol.*, 28: 139-143, 1946.
20. BOURNELL, J. C., AND WORMALL, A. Studies on Bayer 205 (Germanin) and Antrypol. IV. The retention of the drug in the animal body. *Biochem. J.*, 33: 1191-1200, 1939.
21. BOUTARIC, A. Sur la fixation des matières colorantes par les divers constituants du sérum sanguin. *Compt. rend. Soc. de biol.*, 130: 132-134, 1939.
22. BOYER, P. D. The prevention by caprylate of urea and guanidine denaturation of serum albumin. *J. Biol. Chem.*, 158: 715-718, 1945.
23. BOYER, P. D., LUM, F. G., BALLOU, G. A., LUCK, J. M., AND RICE, R. G. The combination of fatty acids and related compounds with serum albumin. I. Stabilization against heat denaturation. *J. Biol. Chem.*, 162: 181-198, 1946.
24. BOYER, P. D., BALLOU, G. A., AND LUCK, J. M. The combination of fatty acids and related compounds with serum albumin. II. Stabilization against urea and guanidine denaturation. *J. Biol. Chem.*, 162: 199-208, 1946.
25. BOYER, P. D., BALLOU, G. A., AND LUCK, J. M. The combination of fatty acids and related compounds with serum albumin. III. The nature and extent of the combination. *J. Biol. Chem.*, 167: 407-424, 1947.
26. BRAUER, R. W., AND PESSOTTI, R. L. The mechanism of the extraction of bromsulphalein from blood plasma by the liver. *Fed. Proc.*, 7: 207, 1948.
27. BRINKHOUS, K. M., SMITH, H. P., WARNER, E. D., AND SPEIGER, W. H. The inhibition of blood clotting. *Am.*

- unidentified substance which acts in conjunction with heparin to prevent the conversion of prothrombin into thrombin *Am J Physiol*, 125: 683-687, 1933
- 28 BRUNER, M The state of cholesterol and the nature of the cholesterol protein complex in pathological body fluids *J Biol Chem*, 108 463-470, 1935
  - 29 BRUNAUER, S, EMMETT, P H, AND TELLER, E Adsorption of gases in multimolecular layers. *J Am Chem Soc*, 60 309-319, 1938
  - 30 BUEBING, E Personal communication
  - 31 BUEBING, E, AND OLIVER GONZALEZ, J Metabolism of *Schistosoma mansoni* Proc 4th International Congress of Tropical Medicine and Malaria Abstracts, pp 80-81, Washington, D C, 1948
  - 32 BULL, H B Adsorption of water vapor by proteins *J Am Chem Soc*, 66: 1499-1507, 1944
  - 33 BUCK, G Die photobiologischen Sensibilisatoren und ihre Eiweisverbindungen. *Biochem Ztschr*, 11 425-540, 1908
  - 34 BUTLER, G C, HARRINGTON, C R, AND YVILL, M E Studies in synthetic immunochemistry 5 Observations on antisera against aspirin protein complexes *Biochem J*, 34: 838-845, 1940
  - 35 CARTER, C E, AND GREENSTEIN, J P Protective effect of thymus nucleate on the heat coagulation of proteins *J Nat. Cancer Inst*, 6 219-223, 1946
  - 36 CARTWRIGHT, G E, BLACK, P, AND WINTHROP, M M Chemical, clinical and immunological studies on the products of human plasma fractionation XXXIX The anemia of infection Studies on the iron binding capacity of serum *J Clin Investigation*, 23 85-98, 1949
  - 37 CHAMUTIN, A, LUDKOW, S, AND MASKET, A V Studies on the calcium protein relationship with the aid of the ultracentrifuge. I Observations on calcium caseinate solutions *J Biol Chem*, 143 737-751, 1942
  - 38 CHAROFF, E, ZIFF, M, AND COHEN, S S Studies on the chemistry of blood coagulation X The reaction between heparin and the thromboplastic factor *J Biol Chem*, 136 257-264, 1940
  - 39 CHAROFF, E, ZIFF, M, AND MOORE, D H Studies on the chemistry of blood coagulation. XII An electrophoretic study of the effect of anticoagulants on human plasma proteins, with remarks on the separation of the heparin complement. *J Biol Chem*, 139 383-405, 1941
  - 40 CHAROFF, E Lipoproteins *Adv in Prot Chem*, 1: 1-24, 1944
  - 41 CHINARD, F P Interactions of quaternary ammonium compounds and proteins A simple method for the rapid estimation of urinary protein concentrations with alkylidimethylbenzylammonium compounds *J Biol Chem*, 176 1430-1447, 1948
  - 42 CLUTTON, R F, HARRINGTON, C R, AND YVILL, M E Studies in synthetic immunochemistry II. Serological investigation of  $\alpha$ - $\beta$ -glucosidotyrosyl derivatives of proteins *Biochem J*, 32 1111-1118 1938.
  - 43 CLUTTON, R F, HARRINGTON, C R, AND YVILL, M E Studies in synthetic immunochemistry III Preparation and antigenic properties of tyrosyl derivatives of proteins, and physiological effects of their antisera *Biochem J*, 32 1119-1132, 1938
  - 44 COHEN, S S, AND CHAROFF, E Studies on the chemistry of blood coagulation. IX. The thromboplastic protein from lungs *J Biol Chem*, 136 243-256 1940
  - 45 CONN, E J, AND EDGALL, J T Proteins, Amino Acids and Peptides as Ions and Dipolar Ions Reinhold Publishing Corporation New York, 688 pp, 1943
  - 46 CONN, E J Blood, blood derivatives and blood substitutes *Proc Am Philosoph Soc*, 83 159-173, 1944
  - 47 CONN, E J, ONCLEY, J L, STRONO, L E, HUGHES, W L, JR, AND ARMSTRONG, S H, JR. The characterization of the protein fractions of human plasma *J Clin Investigation*, 23: 417-432, 1944
  - 48 CONN, E J, STRONO, L E, HUGHES, W L, JR, MULFORD, D J, AARWORTH, J N, MELIN, M, AND TAYLOR, H L Preparation and properties of serum and plasma proteins IV A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids *J Am Chem Soc*, 68 459-475, 1946
  - 49 CONN, E J The chemical specificity of the interaction of diverse human plasma proteins *Blood The Journal of Hematology*, 3: 471-485 1948
  - 50 COOLIDGE, T B Chemistry of the van den Bergh reaction *J Biol Chem*, 132 119-127 1940
  - 51 CUNLIFFE, T B A simple ultrafiltration apparatus *J Biol Chem*, 135 511-542 1940
  - 52 DALE, H H Conditions which are conducive to the production of shock by histamine *Brit J Exper Path.*, 1 103-114, 1920
  - 53 DAVIS, B D, AND WIND, W B, JR. Studies on antibacterial action of sulfonamide drugs II. Correlation of drug activity with binding to plasma proteins *Proc Soc Exper Biol and Med*, 51 283-285, 1942
  - 54 DAVIS, B D Binding of sulfonamides by plasma proteins *Science*, 95 78 1942
  - 55 DAVIS, B D The binding of sulfonamide drugs by plasma proteins. A factor in determining the distribution of drugs in the body *J Clin Investigation* 22 753-762, 1943
  - 56 DAVIS, B D, AND DUBOS, R J Interaction of serum albumin, free and esterified oleic acid and lipase in relation to cultivation of the tubercle bacillus *Arch Biochem*, 11 201-203, 1946
  - 57 DAVIS, B D Physiological significance of the binding of molecules by plasma proteins *Am Scientist*, 34: 611-618 1946
  - 58 DAVIS, B D, AND DUROS, R J The binding of fatty acids by serum albumin, a protective growth factor in bacteriological media *J Exper Med* 86 215-228, 1947
  - 59 DAVIS, B D The binding of chemotherapeutic agents to proteins and its effect on their distribution and activity Chapter in "Evaluation of chemotherapeutic agents Symposium of Section on Microbiology of the N Y Acad of Med" Columbia University Press 1948
  - 60 DE HAAN, J AND VAN CREVELD, S Über die Wechselbeziehungen zwischen Blutplasma und Gewebeflüssig-

- kelten insbesondere Kammerwasser und Cerebrospinalflüssigkeit I Der Zuckergehalt und die Frage des gebundenen Zuckers Biochem Ztschr, 123 190-214 1921
61. DE HAAN J AND VAN CREVELD S Über die Wechselbeziehungen zwischen Blutplasma und Gewebeflüssigkeiten, insbesondere Kammerwasser und Cerebrospinalflüssigkeit II Durchlässigkeit für Fluorescein und Jodsalze Biochem Ztschr, 124 172-181 1921
62. DE HAAN J The renal function as judged by the excretion of vital dye-stuffs J Physiol, 56: 444-450 1922
63. DE HAAN J Die Speicherung saurer Vitalfarbstoffe in den Zellen mit Beziehung auf die Probleme der Phagocytose und der Zellpermeabilität. Arch f ges Physiol, 201 393-401, 1923
64. DEROUX, G Liaison sulfamideprotéine et passage des sulfamidés dans le liquide céphalo-rachidien. Acta biol Belges 3 170-172 1943
65. DEROUX, G Mesure de la diffusion générale du sulfathiazol de la sulfapyridine et de la sulfacetamide. Compt. rend Soc. de biol 135 877-879, 1944
66. DEWEY, H M AND WORMALL, A Studies on Suramin (Antrypol Bayer 205) 5 The combination of the drug with the plasma and other proteins Biochem J, 40 119-124, 1946
67. DOLADILHE, M, AND MAZILLE, M In vitro, un seul constituant de l'édifice protéoséne fixe les matières colorantes. Compt rend Soc. de biol 130 123-129, 1939
68. DOW P Brilliant vital red and T 1824 Comparative studies Fed. Proc. 4 16-17 1945
69. DEINKER, N, GREEN A A, AND HASTINGS, A B Equilibria between calcium and purified globulins J Biol Chem 131 641-647 1939
70. DUGGAN, E L A n LUKE, J M The combination of organic anions with serum albumin IV Stabilization against urea denaturation J Biol Chem, 172. 205-220 1948
71. EAOLE, H The inactivation of penicillins F, G, K and X by human and rabbit serum J Exper Med 85 141-181 1947
72. EAOLE H The varying blood levels afforded by penicillins F, G K and X in rabbits and man. J Exper Med, 85 163-173 1947
73. EAOLE H The therapeutic activity of penicillins F, G K and X in experimental infections with Pneumococcus Type I and Streptococcus pyogenes J Exper Med 85 175-189 1947
74. EAOLE H AND NEWMAN E Renal clearance of penicillins F, G K and X in rabbits and man. J Clin. Investigation 26 903-918 1947
75. EAOLE H AND TUCKER H A The effect of human serum on the dilution bioassay of penicillins G, X and K. J Bact 56 59-62 1948.
76. EARLE D P JR. Renal excretion of sulfamerazine J Clin Investigation 23 914-920, 1944
77. EARLE, D P, JR., AND BRODIE B B The renal excretion of 4-carboxyphenylmethane sulfonanilide (caronsmide) J Pharmacol and Exper Therap 91 250-254 1947
78. EDSELL, J T The plasma proteins and their fractionation. Adv in Protein Chem, 3 381-479 1947
79. EHRLICH P Über den jetzigen Stand der Chemotherapie. Ber Deutsch. Chem Ges 42 17-47 1909
80. EHRLICH P Chemotherapeutics Scientific principles methods and results. Lancet 2 445-451 1913
81. EHRESTRÖM, M CH. Das Adsorptionsvermögen der Plasmaproteine. Acta med. Scandinav 91 191-196, 1937
82. EKENHORN G On the principles of renal function. Acta med Scandinav Suppl 36 717 pp 1931
83. ELDERFIELD R C The chemistry of the cardiac glycosides. Chem. Rev 17 187-249 1935
84. ELSON, K A BOTT P A. AND SHIELDS E H On the excretion of Skiodan Diodrast and Hippuran by the dog Am J Physiol 115 548-555 1936
85. FARAH A On the combination of some cardio-active glycosides with serum proteins. J Pharmacol and Exper Therap 83 143-157 1945
86. FAWAZ, G AND FARAH A. A study of the digitoxin binding power of serum and other soluble tissue proteins of the rabbit. J Pharmacol. and Exper Therap 80- 193-198 1944
87. FELL, N, ROONEY G AND MARSHALL D E Histamine protein complexes Syn thesis and immunologic investigation. I. Histamine azoprotein. J Immunol 47 237-249 1943
88. FERGUSON J H. The action of heparin serum albumin (crystalline) and salmine on blood-clotting mechanisms (in vitro) Am J Physiol., 130 759-770, 1940
89. FISHER, L F AND FISHER, M The Chemistry of Natural Products Related to Phenanthrene. 3rd Edition. Reinhold Publishing Co, New York 1949
90. FRAENKEL-CONRAT H AND COOPER, M The use of dyes for the determination of acid and basic groups in proteins. J Biol Chem 154 239-246 1944
91. FREUNDLICH H Colloid and Capillary Chemistry E. P Dutton and Co New York 1926.
92. FULLER, A T Antibacterial action and chemical constitution in long-chain aliphatic bases. Biochem J 36 548-558 1942.
93. GAILMARD J E. Métabolisme du salicylate de sodium. III. Adsorption in vitro par le sérum sanguin. Bull Soc. chim biol 27 206-211 1945
94. GARDNER, J A AND GAINSBOROUGH H Studies on the cholesterol content of normal human plasma Part II. The attraction of the proteins of plasma for sterols Biochem J 21 141-147 1927
95. GERBER, I E., AND GROSS M The immunological specificity of sulfonamide protein conjugates as demonstrated by anaphylaxis in guinea pigs and the Schwartzman phenomenon in rabbits J Immunol, 48 103-109 1944
96. GIBSON, S T Blood and its derivatives New England J Med. 239 544-556 579-589 1948.
97. GILDEMEISTER, H Porphyrine und Serumelweiss. Ein Beitrag zur Vekelfunktion der Serumelweisskörper Ztschr f d. ges. exper Med., 102: 58-87 1937

98. GILLIOAN, D R. Blood levels of sulfadiazine, sulfamerazine and sulfamethazine in relation to binding in the plasma. *J Pharmacol and Exper Therap*, 79 320-328, 1943
99. GILLMAN, J, GILBERT, C, AND GILLMAN, T A preliminary report on hydrocephalus, epina bifida and other congenital anomalies in the rat produced by trypan blue. The significance of these results in the interpretation of congenital malformations following maternal rubella. *South African J M Sc.*, 13: 47-50, 1948.
100. GOLDBAUM, L R, AND SMITH, P K The binding of barbiturates by human and bovine serum albumin. *Fed. Proc.*, 7: 222, 1948.
101. GOLDRINO, W, CLARKE, R. W AND SMITH, H W The phenol red clearance in normal man *J Clin. Investigation*, 15 221-228, 1936
102. GOLDSTEIN, A Properties and behavior of human plasma cholinesterase To be published.
103. GOLDSTEIN, A Unpublished observations.
104. GOUDAMIT, A, JR., POWER, M H, ANN BOLLMAN, J L The excretion of sulfates by the dog. *Am. J Physiol.*, 125 506-520, 1939
105. GREENBERG, D M, ANN GUNTHER, L On the determination of diffusible and non-diffusible serum calcium. *J Biol Chem*, 85: 491-508, 1930
106. GREENBERG, D M The interaction between the alkali earth cations, particularly calcium, and proteins. *Adv in Protein Chem*, 1 121-151, 1944
107. GREGERSEN, M L, AND GIBSON, J G, 2ND Conditions affecting the absorption spectra of vital dyes in plasma. *Am. J Physiol*, 120: 494-513, 1937
108. GREGERSEN, M I, AND RAWSON, R. A The disappearance of T 1824 and structurally related dyes from the blood stream. *Am J Physiol.*, 138: 698-707, 1943
109. GROLLMAN, A The combination of phenol red and proteins. *J Biol. Chem*, 64 141-160, 1925
110. GROLLMAN, A Ultrafiltration through collodion membranes. *J Gen Physiol.*, 9 813-826, 1926.
111. GUTMAN, A B, AND GUTMAN, E. B Relations of serum calcium to serum albumin and globulins. *J Clin. Investigation*, 16: 903-919, 1937
- 111a. GUTMAN, A B The plasma proteins in disease. *Adv in Protein Chem*, 4: 155-250, 1948.
112. HAARMANN, W, HAUCKMEIER, A, AND LENDLE, L Über die Bindung von Digitalisglykosiden und Digitaloiden an die Eiweißstoffe des Bluteserums. *Arch f exper Path u Pharmakol*, 194 205-216, 1940
113. HAARMANN, W, KOPFMACHER, K., ANN LENDLE, L Bedingungen der Bindung von Digitalisglykosiden an die Serumweißstoffe *Arch. f exper Path u Pharmakol*, 194 229-247 1940
114. HAOOARD, H W An accurate method of determining small amounts of ethyl ether in air, blood, and other fluids, together with a determination of the coefficient of distribution of ether between air and blood at various temperatures. *J Biol Chem*, 55 131-143 1923
115. HALPERN, B, DOLKART, R E, LESH, J B, KUTZ, R. L., DEY, F L., AND WOLNAX, B The relationship of the human plasma proteins in the action and transport of penicillin. To be published, 1949
116. HARTLEY, G S The effect of long-chain salts on indicators The valence-type of indicators and the protein error *Tr Faraday Soc.*, 30: 441-460, 1934
117. HAYASHIDA, A. The "non-solvent space" of the serum and the chlorine bound by the serum protein. *J Biochem (Japan)*, 18: 107-124, 1933
118. HAYMAN, J M, JB The excretion of inorganic sulfates. *J Clin. Investigation*, 11 607-619, 1932
119. HEIM, F Änderung der Acetylcholinwirkung durch Adsorption an Eiweißkörper *Arch. f exper Path. u Pharmakol*, 192 276-291, 1939
120. HEINEMANN, M Distribution of sulfonamide compounds between cells and serum of human blood. *J Clin. Investigation*, 22 29-32, 1943
121. HELLERMAN, L., LINDSAY, A, AND BOVARNICK, M R Flavoenzyme catalysis. Inhibition of d-amino acid oxidase by competition with flavin adenine-dinucleotide of atabrine (quinacrine), quinine, and certain other compounds *J Biol Chem*, 163 553-570 1946
122. HENBY, R. J, BERKMAN, S, ANN HOUSEWRIGHT, R. D Studies on streptomycin. II. Inactivation of streptomycin upon standing in certain culture media and human serum *J Pharmacol. and Exper Therap.* 99: 42-45, 1947
123. HEWITT, L F Combination of proteins with phthalein dyes. *Biochem J*, 21 1305-1313, 1927
124. HEYMANN, H, ANN FIESER, L F Naphthoquinone antimalarials. XIX. Antirespiratory study of protein binding. *J Pharmacol and Exper Therap.*, 94 97-111, 1948.
125. HIATT, E P, AND SUHRIZ, V Renal excretion of cinchona alkaloids and some quaternary base derivatives and their effect on renal hemodynamics *Fed Proc.* 5 46, 1946
126. HIRSCHFELDER, A D, AND WRIGHT H N Studies on the colloid chemistry of antiseptics and chemotherapy I The mode of combination of antiseptic dyes with proteins. *J Pharmacol. and Exper Therap*, 38 411-431, 1930
127. HOKKSTRA, R. A Das Verhalten von Digitalisglykosiden in Blut und Gewebeflüssigkeit. *Arch. f exper Path u Pharmakol*, 162 649-662, 1931
128. HOKKSTRA, R. A Das Eindringen der Digitalisglykoside in die Organe. *Arch. f exper Path u Pharmakol.*, 162 663-684, 1931
129. HOOKER, S. B, ANN BOYD, W C Antibodies to strychnine. *J Immunol.* 38 479-490, 1940
130. HOOVER, C F, ANN BLANKENHORN, M A Dissociated jaundice. *Arch Int. Med* 18 289-303, 1916.
131. HUGHES, W L, JR. An albumin fraction isolated from human plasma as a crystalline mercuric salt. *J Am. Chem Soc.*, 69: 1836-1837, 1947
132. JACKSON, E L. Personal communication.

133. JACOBS, L. B. The reaction of heparin with proteins and complex bases. *Biochem. J.*, 37 189-195 1943
134. KIMMO, J. AND WESELMANN, H. Die Bindungsverhältnisse zwischen Bluteiweisskörper und Sulfonamiden. *Arch. f. Dermat. u. Syph.* 182 436-451, 1942.
135. KLOPFROCK, F. Ueber den Einfluss von Heparin und Germanin auf Immunreaktionen. *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 75 348-354, 1932.
136. KLOTZ, I. M. LONGFELLOW, J. M. ANN JOHNSON, O. H. Interactions between proteins and azosulfonamides. *Science*, 104 264, 1946.
137. KLOTZ, I. M. The application of the law of mass action to binding by proteins. *Interactions with calcium*. *Arch. Biochem.*, 9: 109-117, 1946.
138. KLOTZ, I. M., WALKER, F. M. ANN PIVAN, R. B. The binding of organic ions by proteins. *J. Am. Chem. Soc.*, 68 1485-1490 1946.
139. KLOTZ, I. M. Spectrophotometric investigations of the interactions of proteins with organic ions. *J. Am. Chem. Soc.*, 68 2299-2304, 1946
140. KLOTZ, I. M., AND WALKER, F. M. The binding of organic ions by proteins. Charge and pH effects. *J. Am. Chem. Soc.*, 69 1600-1612 1947
- 140a. KLOTZ, I. M., TRIWOSH, H., AND WALKER, F. M. The binding of organic ions by proteins. Competition phenomena and denaturation effects. *J. Am. Chem. Soc.* 70: 2235-2241 1948.
141. KLOTZ, I. M., ANN URQUHART, J. M. The combination of adenine, adenosine and adenylic acid with serum albumin. *J. Biol. Chem.* 173 21-24, 1948.
142. KLOTZ, I. M. Personal communication.
143. KOCIAN, V. Über die Schntzwirkung des Germanins (Bayer 203) auf die Koagulation der Bluteiweissstoffe. *Arch. f. exper. Path. u. Pharmacol.* 182. 313-316, 1936.
144. KOELLE, G. B. Protection of cholinesterase against irreversible inactivation by di isopropyl fluorophosphate in vitro. *J. Pharmacol. and Exper. Therap.* 83 232-237, 1946.
145. LANGMUIR, I. The constitution and fundamental properties of solids and liquids. Part I. Solids. *J. Am. Chem. Soc.*, 38: 2221-2295 1916.
- 145a. LANGMUIR, I. The constitution and fundamental properties of solids and liquids. Part II. Liquids. *J. Am. Chem. Soc.*, 39 1848-1906 1917
146. LAPORTA, M. Circa l'adsorbimento dei sali biliari sulle proteine del siero. *Arch. di. sc. biol.* 26 15-31, 1940
147. LAVIETTES, P. H. Anaerobic ultrafiltration. *J. Biol. Chem.*, 120 257-275 1937
148. LECOMTE DU NOÛT, P. Surface tension of serum. III. Recovery after lowering by surface-active substances. *J. Exper. Med.* 36 115-134, 1922
149. LECOMTE DU NOÛT, P. Sur la capacité d'adsorption des protéines du sérum vis-à-vis des sels biliaires. *Compt. rend. Soc. de biol.* 99: 1097-1099 1923
150. LENDLE, L. AND FRUCH, P. Über die Bindung der Digitaliskörper an die Eiweissstoffe des Blutes. *Arch. f. exper. Path. u. Pharmacol.* 177 550-563 1935
151. LEVINE, R., WOLFSON, W. Q., AND LENEL, R. Concentration and transport of true urate in the plasma of the azotemic chicken. *Am. J. Physiol.* 151 185-191, 1947
152. LINDEWEAVER, H., AND BORK, D. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* 56 658-666 1934
153. LOISELEUX, J. AND COLLIARD, R. Sur l'adsorption des polypeptides par les protéides du plasma sanguin. *Compt. rend. Acad. d. sc.* 205 261-263 1937
154. LONTIE, R. MORRISON, P. K. EOELROCH, H. AND EDSELL, J. T. Light scattering in solutions of serum albumin and  $\gamma$ -globulin. Presented at Am. Chem. Soc., Division of Biological Chemistry August 31 1943
155. LUBROW, S. CHANTIN, A., AND MASKET, A. V. Studies on the calcium protein relationship with the aid of the ultracentrifuge. II. Observations on serum. *J. Biol. Chem.*, 143 733-761 1942.
156. LUNDBORN, H. P. ELAM, D. W., AND O'CONNELL, R. A. Electrophoretic study of the action of alkylbenzene sulfonate detergents on egg albumin. *J. Biol. Chem.* 149: 183-193 1943
157. LUNOQUIST, F. Renal tubular secretion of sulfonamides and p-aminobenzoic acid. *Acta Pharm. et Toxicol.* 1 307-324 1945
158. MACHEBOEUF, M. AND VICOENTIN, M. Étude des combinaisons entre protéides et cuivre prenant naissance en milieu alcalin. *Compt. rend. Acad. d. sc.* 218 977-978 1944
159. MALINOW, M. R., ANN KOBSON, W. An experimental method for obtaining an ultrafiltrate of the blood. *J. Lab. and Clin. Med.* 32 461-471, 1947
160. MALLOY, H. T. AND EVELYN, K. A. The determination of bilirubin with the photoelectric colorimeter. *J. Biol. Chem.*, 119: 481-490 1937
161. MARSHALL, E. K. JR. AND VICKERS, J. L. The mechanism of the elimination of phenolsulfophtalein by the kidney—a proof of secretion by the convoluted tubules. *Bull. Johns Hopkins Hosp.* 34 1-7 1923
162. MARSHALL, E. K. JR. The secretion of phenol red by the mammalian kidney. *Am. J. Physiol.*, 99 77-80 1931
163. MARSHALL, E. K. JR., AND DEARBORN, E. H. The relation of the plasma concentration of quinine to its antimalarial activity. *J. Pharmacol. and Exper. Therap.* 83 142-153 1946
164. MARTIN, N. H. Bilirubin-serum protein complexes. *Biochem. J.* 42 No. 1 Proceedings p. xv, 1948.
165. MARTIN, N. H. Preparation and properties of serum and plasma proteins. XIX. Interactions with bilirubin. *J. Am. Chem. Soc.* 71 In press 1949
166. MATHEWS, A. P. Adsorption. *Physiol. Rev.*, 1 553-597 1921
167. MAYER, M. ANN ZEISS, H. Über die Wirksamkeit des Serums mit 'Bayer 203' vorbehandelter Kaninchen. *Arch. f. Schiffs u. Tropen Hyg.* 25 259-271, 1921

168. MAYER, M, ZEISS, H, GRENDA, G, AND HALBERKANN, J Weitere Beobachtungen über das Verhalten des neuen Trypanosomenheilmittels "Bayer 203" im Blute. *Arch f Schiffs- u. Tropen-Hyg.*, 26 140-146 1932
169. McLEAN, F C, AND HASTINGS, A B The state of calcium in the fluids of the body I. The conditions affecting the ionization of calcium *J Biol Chem*, 103 285-322, 1935
170. MELLANDER O Kataporetische Untersuchungen über die Bindungsverhältnisse des Cholesterins im Blutserum *Biochem Ztschr*, 277 305-313, 1935
- 170a. MEYER, K, AND ALEXANDER, M E Versuche über die anaphylaktogene Wirkung kristalloider Substanzen. *Biochem Ztschr*, 146: 217-220, 1924
171. MEYER, K. Mucoids and glycoproteins. *Adv in Protein Chem*, 2: 249-275, 1945.
172. MICHAELIS, L, AND GRANICK S Metachromasy of basic dyestuffs. *J Am. Chem. Soc.*, 67 1212-1219, 1945.
173. MOORE, B, AND ROAF, H E On certain physical and chemical properties of solutions of chloroform in water, saline serum, and hemoglobin A contribution to the chemistry of anaesthesia. Preliminary communication. *Proc Roy Soc, London*, B, 73 382-412 1934.
174. MOORE, B, AND ROAF, H E On certain physical and chemical properties of solutions of chloroform and other anaesthetics. A contribution to the chemistry of anaesthesia. Second communication. *Proc. Roy Soc, London*, B, 77: 88-102, 1935
175. MORRISON P R, EDGALL, J T, AND MILLER, S G Preparation and properties of serum and plasma proteins XVIII The separation of purified fibrinogen from Fraction I of human plasma. *J Am. Chem. Soc.*, 70 3100-3108 1948.
176. MULINOS M G AND SCHLESINGER, E A contribution to drug allergy antipyrine. *Proc. Soc. Exper Biol. and Med*, 35 305-307, 1937
177. MULINOS, M G, SCHLESINGER, E, AND STEIN, D B Antigenic properties of some azo compounds of serum albumin and serum globulin. *Proc. Soc. Exper Biol and Med*, 37 583-587, 1938.
178. NORTHRUP J H, AND KUNITZ, M The combination of salts and proteins. *I J Gen Physiol* 7 25-38 1924.
179. OLIVER J, AND DOVOLAS E Biologic reactions of arsenphenamin V Its reactions with plasma proteins and certain hydrophilic colloids and the relation of these processes to the phenomenon of protection. *Arch. Dermat. and Syphl*, 7: 778-799, 1933
180. ONCLEY J L, MELIN, M RICHERT, D A., CAMERON J W, AND GROSS P M, JR. Preparation and properties of serum and plasma proteins XII. The separation of the antibodies isocagglutinins, prothrombin, plasminogen, and X protein into subfractions of human plasma. *J Am Chem. Soc.*, in press, 1949
181. ONCLEY J L, AND GORN, F R N Unpublished observations
182. OPPENHEIMER, E Zur Frage der Fixation der Digitalskörner im tierischen Organismus und besonders deren Verhalten zum Blut. *Biochem Ztschr*, 55: 134-152 1913
183. OTCUTT, F S AND SKEVENS M H The solubility coefficients of cyclopropane for water, oils and human blood *J Pharmacol and Exper Therap*, 59 206-210, 1937
184. OSBORNE W A Intracellular colloidal salts *J Physiol.*, 34 84-92 1906.
185. PAOT M, AND VITTO, C Recherche de l'existence d'une combinaison sérum para-amino-phénylsulfamide par l'étude des indices de réfraction. *Compt. rend Soc. de biol* 140: 227-233 1946.
186. PAK C Versuche über den Übertritt chemischer Substanzen aus der Gefäßbahn in die Gewebe. *Arch f exper Path u Pharmacol*, 111 42-59, 1926
187. PAULI, W, AND SCHÖY M Untersuchungen an elektrolytfreien, wasserlöslichen Proteinen III. Mitteilung. Sulfonweissverbindungen (ZnCl<sub>2</sub>) *Biochem Ztschr* 153 233-234, 1924
188. PAULING, L, CAMPBELL D H, AND PRESSMAN, D The nature of the forces between antigen and antibody and of the precipitation reaction. *Physiol Rev*, 23: 203-219 1943
189. PEARSON P B, AND CATCHPOLE, H R Correlated studies of the partition of calcium and inorganic phosphorus in the blood sera of Equidae. *Am J Physiol* 115: 90-93, 1936
190. PETRUNKIN A, AND PETRUNKIN M Combination of gelatin with some organic bases. *J Gen Physiol*, 11: 101-110 1927
191. PFAFF W, AND HEROLD, W Grundlagen einer neuen Therapieforchung der Tuberkulose. Leipzig, 1937 Cited by Bennhold (11)
192. PITTS, R F The excretion of phenol red by the chicken. *J Cell and Comp Physiol* 11 99-115 1938
193. PLATTNER, F Zur Frage der Ausscheidung saurer Farbstoffe durch die Leber *Arch f d ges Physiol.*, 206: 91-100 1924
194. PUTNAM, F W, AND NEURATH H Complex formation between synthetic detergents and proteins *J Biol. Chem*, 150 283-294, 1943
195. PUTNAM, F W, AND NEURATH, H Interaction between proteins and synthetic detergents. II Electrophoretic analysis of serum albumin-sodium dodecyl sulfate mixtures. *J Biol Chem* 159 193-209 1945
196. PUTNAM, F W The interactions of proteins and synthetic detergents. *Adv in Protein Chem*, 4 70-122, 1948.
197. QUICK A J The normal antithrombin of the blood and its relation to heparin *Am J Physiol*, 123: 712-719 1938.
198. RAKOFF, A E, PASCHKIS, K E, AND CANTAROW, A. Conjugated estrogens in human pregnancy serum *Am J Obst and Gynec.* 46 856-860 1943
199. RAKOVIN, M A Über die Einwirkung von Anilinfarbstoffen auf Albumin Casein und Gelatine. *Biochem. Ztschr* 192 167-171 1928.
200. RATH, C E AND FINCH, C A Chemical clinical, and immunological studies on the products of human plasma fractionation XXXVIII Serum iron transport. Measurement of iron-binding capacity of serum man *J Clin Investigation*, 28 79-85, 1949



200. RAWLINS, L. M. C., AND SCHMIDT C. L. A. The mode of combination between certain dyes and gelatin granules. *J Biol Chem.*, 88 271-284, 1930
201. RAWSON, R. A. The binding of T-1824 and structurally related diazo dyes by the plasma proteins. *Am J Physiol.*, 138 703-717, 1943
202. RICHARDS A. N., AND WALKER, A. M. Quantitative studies of the glomerular elimination of phenol red and indigo carmine in frogs. *J Biol Chem.*, 87 479-493 1930
203. RICHARDSON A. P., MILLER, L., SCHUMACHER C., JAMBOR, W., PANSY, F., ANN LAPIDES, D. Physiological disposition of penicillins G and K in dogs. *Proc. Soc. Exper Biol and Med* 63 514-519, 1946.
204. RIGGS, D. S. AND MAN, E. B. A permanganate acid ashing micromethod for iodine determinations. Values in blood of normal subjects. *J Biol. Chem.*, 134 193-211, 1940
205. RIGGS, D. S. Serum protein bound iodine as a diagnostic aid. *Tr Am. A. Study Gaster*, p 137-144, 1947
206. RIGGS, D. S. Personal communication.
207. ROBERTS, S., AND SKEGG, C. M. The nature of circulating estrogen Lipoprotein bound estrogen in human plasma. *Endocrinology*, 39 183-187, 1946.
208. ROBINSON, H. W. ANN HOONKY, C. G. The influence of serum proteins on the spectrophotometric absorption curve of phenol red in a phosphate buffer mixture. *J Biol Chem*, 137 239-254, 1941
209. RONNEY G. AND FELL, N. Histamine-protein complexes: Synthesis and immunologic investigation. II.  $\beta$ -(5-imidazolyl) ethyl carbamido protein. *J Immunol.*, 47: 251-259, 1943
210. ROSENFELD M. Cited by COHN (49)
211. ROSENTHAL, S. M., AND WHITE, E. C. Studies in hepatic function. VI. A. The pharmacological behavior of certain phthalein dyes. B. The value of selected phthalein compounds in the estimation of hepatic function. *J Pharmacol and Exper Therap* 24 265-268 1924
212. ROSENTHAL, S. M. The liberation of adsorbed substances from proteins. A function of the bile salts. I. Preliminary report. *J Pharmacol. and Exper Therap.* 25 419-457, 1925
213. ROSENTHAL S. M. Studies upon the combining power of proteins with rose bengal. *J Pharmacol and Exper Therap* 29- 521-532, 1924.
214. ROSENTHAL, S. M. The liberation of adsorbed substances from the proteins. II. The effect of addition of sodium cleats to whole blood upon the non protein nitrogen in blood filtrates *J Biol. Chem.* 70 129-133, 1925.
215. ROTHLEN, E. The glucoside binding action of different fractions of human serum and plasma proteins. Unpublished manuscript, 1943.
216. ROTHLEN E. Das Blut und seine Funktion für den Transport und die Verteilung von Wirkstoffen. *Vierteljahrsschr d Naturforsch. Ges., Zürich* 90- 193-214 1945
217. SALTZ, W. T., WHITE, W. F., AND MCHAY, E. A. The lymphatic conveyance of thyroid hormone. *Fed. Proc.*, 5 90 1946
218. SAMSON I. W., AND GÖTZ, H. Körperprotein und Arzneimittelallergie. *Ztschr f d. ges. exper Med.*, 52: 121-135, 1926.
219. SAROFF H. A., ANN MARK, H. J. Personal communication.
220. SCATCHARD G., BATCHELDER, A. C., AND BROWN A. Preparation and properties of serum and plasma proteins. VI. Osmotic equilibria in solutions of serum albumin and sodium chloride. *J Am. Chem. Soc.*, 68: 2320-2329, 1946.
221. SCATCHARD G., AND BLACK, E. S. The effect of salts on the isomeric and isoelectric points of proteins *J Phys and Colloid Chem*, in press 1949
- 221a. SCATCHARD G. The attractions of proteins for small molecules and ions. Conference on Molecular Interaction April 9-10 1948. *Ann N Y Acad. Sci* in press.
222. SCATCHARD G. SCHEINBERG I. H., AND ARMSTRONG S. H., JR. *J Am. Chem. Soc.* to be published.
223. SHADE, A. L., AND CAROLINE, L. An iron binding component in human blood plasma. *Science*, 104. 340-341 1948.
224. SCHMIDT, C. L. A., Editor. The Chemistry of the Amino Acids and Proteins. Charles C Thomas Spring field Illinois 1290 pp, 1944.
225. SCHÖVOLLER, G. Die Bindung von Prontosil an die Blutleisskörper. *Klin. Wchnschr*, 19 790-791, 1940
226. SCHUBERT R. Verhalten wasserlöslicher Vitamine gegenüber den Serumleisskörpern mit besonderer Berücksichtigung des Transportproblems. *Ztschr f Vitaminforsch*, 19 119-179, 1947
227. SCHULTEN H. Über die Harnbildung in der Frochmiere. III. Mitteilung. Die Ausscheidung von Säurefarbstoffen durch die überlebende Frochmiere. *Arch f d. ges. Physiol*, 203 1-15 1925
228. SRI S. Über das Verhalten von Lösungen einiger Blamutyltartrate bzw deren Mischungen mit Blutserum bei der Ultrafiltration. *Arch. f Dermat. u. Syph* 146 43-54 1924
229. SHANNON J. A. The excretion of phenol red by the dog. *Am. J Physiol.*, 113 602-610 1935
230. SHEPPARD S. E. AND GEORGE A. L. Amphipathic character of proteins and certain lyophilic colloids as indicated by absorption spectra of dyes. *J Chem. Physics* 13 63-65 1945
231. SIENKOWICZ, S. S., AND CLARK A. J. A note on the passage of trypan blue from the blood stream into body fluids. *J Pharmacol and Exper Therap* 24 301-304 1924
232. SINGER E. Serological protection against arsenic compounds. *Australian J Exper Biol and M Sc* 20 209-212 1942.
233. SINGER, M., AND MORRISON P. R. The influence of pH dye and salt concentration on the dye binding of modified and unmodified fibrin. *J Biol. Chem* 175 133-145 1948.
234. SKEGGS L. T., JR. AND LEONARDS J. R. Studies on an artificial kidney I. Preliminary results with a new type of continuous dialyzer. *Science* 103 212-213 1943.

- 235 SMITH, H W The Physiology of the Kidney Oxford University Press, New York, 134 pp, 1937
- 236 SMITH, H W, FINKELSTEIN, N, ALMINOBA, L, CRAWFORD, B, AND GRABER, M The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J Clin. Investigation*, 24: 388-404, 1945
- 237 SMITH, P K., GLEASON, H L, STOLL, C G, AND OORZALEK, S. Studies on the pharmacology of salicylates. *J Pharmacol and Exper Therap*, 87 237-253, 1945
- 238 SMITH R. M., JOSELYN, D A., GRUBBIT, O M., McLEAN, I. W., JR., PENNER, M A., AND EHRLICH, J Chloromycetin Biological studies *J Bact.*, 55 425-448, 1948
- 239 SMITH, W W, AND SMITH, H W Protein binding of phenol red, diodrast and other substances in plasma. *J Biol Chem*, 124 107-113, 1938.
- 240 BÖRENSEN, S P L Enzymstudieo (Fortsetzuog) II Mitteilung. Über die Messung und die Bedeutung der Wasserstoffionkonzentration bei enzymatischen Prozessen. B Colorimetrische Messungen. *Biochem Ztschr*, 21: 201-304, 1909
- 241 SPINKS, A The persistence in the blood stream of some compounds related to suramin. *Biochem. J*, 42, 109-116, 1948
- 242 STEARN, A E. Stoichiometrical relations in the reactions between dye, nucleic acid, and gelatin. *J Biol Chem*, 91: 323-331, 1931
- 243 STEINHARDT, J Participation of anions in the combination of proteins with acids. *Ann. N Y Acad. Sc.*, 41: 287-320 1941
- 244 STENHAAGEN, E, AND TORELL, T Electrophoretic properties of thymonucleic acid. *Tr. Faraday Soc.*, 35: 743-750, 1939
- 245 STORM VAN LEEUWEN, W On the influence of colloids on the action of non-colloidal drugs. I. *J Pharmacol. and Exper Therap*, 17 1-20, 1921
- 246 STORM VAN LEEUWEN, W, AND ZEIJNER, J On the influence of colloids on the action of non-colloidal drugs. II. *J Pharmacol and Exper Therap*, 17 121-127, 1921
- 247 STORM VAN LEEUWEN, W, AND VON SZENT GYÖRGI, A On the influence of colloids on the action of non colloidal drugs. IV. *J Pharmacol. and Exper Therap*, 18 271-291, 1921
- 248 STORM VAN LEEUWEN, W On sensitiveness to drugs in animals and men. *J Pharmacol and Exper Therap.*, 24 13-19, 1924
- 249 STORM VAN LEEUWEN, W On antagonism of drugs. *J Pharmacol. and Exper Therap*, 24 21-24, 1924
- 250 STORM VAN LEEUWEN A possible explanation for certain cases of hypersensitiveness to drugs in men. *J Pharmacol and Exper Therap*, 24 25-32, 1924
- 251 STORM VAN LEEUWEN, W, AND DREIMAL, H Über die Blutungs-fähigkeit des Blutes für Salizylsäure in Zusammenhang mit Überempfindlichkeit gegen Salizylsäure. *Arch f exper Path u. Pharmacol.*, 102: 218-225, 1924.
- 251a. SUROKOR, D M, KOECHLIN, B A, AND STRONG, L E Chemical, clinical, and immunoological studies on the products of human plasma fractionation. XXV. VII The metal combining globulin of human plasma. *J Clin Investigation*, 28 73-78, 1949
252. SURGENOR, D M, STRONG, L E., TAYLOR, H L., GORDON, R. S., JR., AND GISSON, D M Preparation and properties of serum and plasma proteins. XX. The separation of cholinesterase, mucoprotein and metal combining protein into subfractions of human plasma. *J Am Chem Soc.*, in press, 1949
- 253 SZEGO, C M, AND ROBERTS, S The nature of circulating estrogen. *Proc. Soc. Exper Biol. and Med.*, 61: 161-164, 1946.
254. TAGGART, J V, AND EARLE, D P, JR. Cited by DAVIS (59)
- 255 TAYEAU, F, AND ROLLAND E. Le pouvoir adsorbant des protéines vis-à-vis des sels biliaires. *Bull. Soc. chim. biol*, 29 108-111, 1947
- 256 TAYLOR F H L, AND YOUNG, A G Biochemical studies of mercury compounds. I. The effect of acids, bases, salts and blood serum on the diffusion of mercury compounds in vitro. *J Pharmacol. and Exper Therap*, 38 217-229, 1930
- 257 TEAGUE, R S, AND PERDUE, M The effect of ether and dual urethane anesthesia on the passage of sulfathiazole into the cerebrospinal fluid. *J Pharmacol and Exper Therap*, 92 277-282, 1948.
258. TEPPERMAN, J, RAKILTEN, N, VALLEY, G, AND LYON, E W Inactivation of penicillin G and K by liver and kidney. *Science*, 105 18-19, 1947
- 259 TERST, J D, AND LUCK J M The combination of organic anions with serum albumin. VI. Quantitative studies by equilibrium dialysis. *J Biol Chem*, 174: 653-661, 1948.
- 260 THIMANN K. V, AND ROTHSCHELD, J Unpublished observations
- 261 TOMPSETT R, SHULTZ S, AND McDERMOTT W Relation of protein binding to the pharmacology and antibacterial activity of penicillins X, G, dihydro-F, and K. *J Bact*, 53: 581-595, 1947
- 262 TOMPSETT, R. Unpublished observations. Cited by DAVIS (59)
- 263 TRENNLENBURG P, AND GOEBEL, W Totale nach Entfernung der Epithelkörperchen und Calciummangel im Blute. *Arch f exper Path u Pharmacol*, 89 171-199, 1921
264. VAN DYKE, H B, TUPIKOVA, N A, CHOW B F, AND WALKER, H A. The pharmacological behavior of some derivatives of sulfadiazine. *J Pharmacol and Exper Therap*, 83 203-212, 1945
- 265 VON JANCsó, N Die Untersuchung der Funktion des Retikuloendothels mit Durchströmungsversuchen. *Ztschr f d ges. exper Med* 64 256-267, 1929
- 266 WAT, E L, SMITH, P K, HOWIE, D L WEISS R. AND SWANSON, R. The absorption, distribution, excretion and fate of para-aminosalicylic acid. *J Pharmacol and Exper Therap.*, 93: 368-383, 1948.

- 257 WEIL, A. J. The Wassermann antigen and related "alcohol soluble" antigens. *Bact. Rev.* 5 293-330, 1941.
- 263 WENZEL, W. B. The influence of naphthoquinones upon the respiratory and carbohydrate metabolism of malarial parasites. *Fed. Proc.*, 5 406-407, 1946.
- 269 WENT, S., AND KESZTÖCS, L. Das Antiadrenalin. I. Mitteilung Synthetische Herstellung eines Adrenalin-antigens. Aminoadrenalin und Adrenalin azoproteine. *Arch. f. exper. Path. u. Pharmacol.*, 193 609-614, 1939
- 270 WENT, S., KESZTÖCS, L., AND SZILÁGYI, T. Weitere Untersuchungen über die physiologische Wirkung der Adrenalinazoprotein Antikörper. *Arch. f. exper. Path. u. Pharmacol.* 202 143-149 1943
- 271 WIDENBAUER, F. Über Eiweiß-gebundenes Calcium im Blutserum. *Klin. Wchnschr.* 22 63 1943
- 272 WIDENBAUER, F. Über das Eiweiß-gebundene Calcium bei der Blutgerinnung. *Klin. Wchnschr.* 22 320 1943
- 273 WITH, T. K. Spectral absorption of bilirubin. Measurements in pure aqueous solutions and in solutions containing human serum. *Acta. physiol. Scandinav.* 10 172-180, 1945
- 274 WÖHLISCH, E., AND GRÖNING, W. Über die Antithrombinwirkung der Serumproteine und ihre Beziehung zur Metathrombinbildung. *Biochem. Ztschr.* 305 183-192, 1940
- 275 WÖHLISCH, E., AND KÖHLER, V. Zur Frage der gerinnungsphysiologischen Bedeutung der Serumproteine. *Naturwissensch.* 28, 550-551, 1940.
- 276 WOLFSON, W. Q., LEVINE, R., AND TINSLEY, M. The transport and excretion of uric acid in man. I. True uric acid in normal cerebrospinal fluid, in plasma, and in ultrafiltrates of plasma. *J. Clin. Investigation*, 26 991-994, 1947
- 277 WOLFSON, W. Q., COHEN, C., LEVINE, R., AND HUNBLESTON, B. The transport and excretion of uric acid in man. III. Physiological significance of the uricosuric effect of caronamide. *Am. J. Med.*, 4 774 1949.
- 278 WOLFSON, W. Q. Personal communication.
- 279 WUHRMANN, F., AND WUNDERLY, CH. Die Bluteiweißkörper in der klinischen Medizin auf Grund der fraktionierten Aussalzung der Elektrophorese und der Nephelometrie. *Helvet. med. acta. Suppl.* 10 138 pp 1943
- 280 ZIFF, M., AND CHARGAFF, E. Studies on the chemistry of blood coagulation. XI. The mode of action of heparin. *J. Biol. Chem.*, 136 639-693 1940

# ANTICHOLINESTERASE DRUGS

GEORGE B KOELLE AND ALFRED GILMAN

*From the Wilmer Institute, Johns Hopkins Hospital and the Department of Pharmacology, College of Physicians and Surgeons, Columbia University*

**INTRODUCTION** The pharmacological actions of "anti-cholinesterase drugs" can be considered only after certain limitations have been placed on the definition of the compounds in this category. The first limitation is concerned with the nature of the enzyme inhibited. The term "cholinesterase" (ChE), originally applied to any acetylcholine (ACh)-splitting enzyme of the blood or tissues, has been shown to be too inclusive. There are several enzymes which hydrolyze ACh, and their substrate specificities and susceptibilities to inhibition by different agents vary greatly. Moreover, the physiological significance of many of them in respect to the hydrolysis of ACh is highly questionable. Unfortunately, tests of the anti-ChE activity of drugs have been conducted largely with the ACh-splitting enzyme of mammalian plasma rather than with tissue enzymes. Secondly, literally hundreds of compounds have been reported as more or less potent inhibitors of one or another type of ChE, and many of their pharmacological actions have been attributed to this property. It is generally a wise procedure to adhere to the old pharmacological dictum that no drug has a single action. Undoubtedly, the property of many of the drugs mentioned below to inhibit ChE constitutes a relatively unimportant side-action. On the other hand, potent and specific inhibitors of ChE may exert direct actions on effector cells. An inhibitor combines with an enzyme by virtue of an affinity between certain active groups of the enzyme and inhibitor molecules. Inasmuch as ACh is able to combine with either the enzyme molecule or some "receptor group" of the effector cells, it is likely that enzyme and cell receptor have certain chemical or physical properties in common. It is therefore reasonable to assume that an enzyme inhibitor might also combine with the receptor groups of the effector cells, leading either to the initiation of a response similar to that evoked by ACh or to cholinergic blockade. Specific examples of drugs which appear to act by these mechanisms are cited below.

The present review will be largely confined to a discussion of the actions *in vitro* and *in vivo* of a limited group of compounds which are potent inhibitors of specific ChE, namely, the alkaloids physostigmine, neostigmine and their congeners, and an expanding group of alkyl phosphates. For orientation and background the types of ChEs and their distribution will first be considered.

Any discussion of anti-ChE drugs is bound to touch upon the controversial issues of the rôle of ACh in synaptic transmission and axonal conduction. Obviously this subject cannot be adequately covered. However, an attempt will be made to review the major contributions of studies on the anticholinesterases to the theory of chemical mediation of the nerve impulse.

**CLASSIFICATION OF CHOLINESTERASE ENZYMES** In 1926, Loewi and Navratil

(196) discovered an enzyme in the frog heart which was capable of inactivating the "vagus-substance" and ACh. Stedman and co-workers (288), in the course of their studies of the ACh-splitting activity of horse serum, suggested the term "choline-esterase" to describe enzymes of this type. Since then, a massive literature has accumulated on the properties, distribution and differentiating characteristics of the ChE enzymes. A consideration of certain fundamental principles which have emerged from these investigations is necessary for an understanding of the present concept of the mechanism of action of the anti-ChE drugs. Although all controversial points have not been settled and the investigative possibilities of the subject are by no means exhausted, certain facts are now apparent which permit studies of these drugs to be conducted on a sounder basis than previously.

It was noted as early as 1928, by Galchr and Plattner (114), that the ChE splitting activities of serum and whole blood differed, and that at low substrate concentrations the latter was the more active. Therefore, they suggested that the corpuscles were probably primarily responsible for the hydrolysis of ACh in the bloodstream. However, they attributed the activity in this respect to a non-specific surface action rather than to an enzyme. Following extensive studies of the properties of serum ChE of different species by Stedman and co-workers (76, 288) and by Glick (118, 119, 120) and others, Alles and Hawes (5) confirmed the earlier observation of the greater ACh-splitting capacity of erythrocytes at low substrate concentrations, but they considered the action to be due to the presence of a ChE in the red cells. The erythrocyte ChE was further differentiated from that of serum by its ability to hydrolyze acetyl- $\beta$ -methylcholine, and by its greater sensitivity to changes in pH and salt concentration. Subsequent investigations of the substrate specificities of ACh-splitting enzymes from various sources and their sensitivities to different inhibitors (253) led Mendel and Rudney (216, 217, 218) to propose a general classification of enzymes of this type. Included under the term "pseudo-ChE" were the enzymes from serum, pancreas and other tissues which hydrolyzed ACh, benzoylcholine and several non-choline esters (tributyrin, tripropionin, methyl butyrate) but not acetyl- $\beta$ -methylcholine, and which exhibited maximal activity in the presence of high concentrations of ACh. The term "true-ChE" was reserved for the enzymes of erythrocytes and nervous tissue which acted only on choline esters, including acetyl- $\beta$ -methylcholine but not benzoylcholine, and which were inhibited by high concentrations of ACh. As a method for estimating quantitatively the presence of true-ChE, pseudo-ChE and other esterases in tissues, they suggested the determination of the activities against acetyl- $\beta$ -methylcholine and benzoylcholine, and of the ratio of the rates of hydrolysis of ACh at concentrations of 0.06 and 0.0006 M. It was found that by treating true-ChE with positively charged colloids, such as protamine, the optimal substrate concentration could be raised to a higher level without modification of the substrate specificity. This effect could be reversed by subsequent treatment with a negatively charged colloid (219).

The above classification was criticized by several authors (121, 186), and Nachmansohn and Rothenberg (238, 239) proposed that the enzyme of eryth-

rocytes and nervous tissue be designated "specific-ChE," on the basis of its relative specificity for ACh, to distinguish it from the numerous other ACh-splitting esterases found in serum and various tissues "Specific-ChE" was characterized as exhibiting a definite pattern of substrate relationships, in that it (a) split ACh at a higher rate than any other substrate, the rates of hydrolysis of other choline esters decreasing with increasing chain length of the acyl group, (b) hydrolyzed acetyl- $\beta$ -methylcholine, but at a lower rate than ACh, and (c) failed to attack benzoylcholine, carbaminoylecholine or esters of simple alcohols. Although they confirmed the inhibition of specific ChE by high concentrations of ACh, they found the optimal level to be considerably higher than that reported by Mendel and Rudney, and attributed the discrepancy to the short periods of determination employed by the previous authors.

The use of specific inhibitors has also been suggested as a method for distinguishing between specific-ChE and related enzymes. Inhibition by neostigmine was one of the criteria used by Easson and Stedman (76) for this purpose, but it has been shown since that this compound is highly potent against nearly all types of ChE. Zeller and Bissegger (322) found that percaine inhibited selectively the ChE of serum, caffeine that of erythrocytes and brain, while morphine was a relatively potent inhibitor of both types. Di-isopropyl fluorophosphate (DFP) has been shown to inhibit the ChE of the serum of most species in concentrations far lower than those required to inhibit erythrocyte or brain ChE (147, 211). Another chemical warfare agent,  $\beta$ - $\beta'$ -dichlor-diethyl-N-methylamine (DDM) was found by Adams and Thompson (2) to exhibit the reverse type of selective inhibition. These authors proposed the use of the  $I_{50}$  (molar concentration producing 50 per cent inhibition) ratio,  $I_{50}\text{DDM}/I_{50}\text{DFP}$ , for distinguishing between the two types, the specific-ChEs having relatively low ratios and the non-specific types far higher ratios (e.g.,  $6.7 \times 10^2$  for human brain,  $4.0 \times 10^3$  for human plasma).

Numerous exceptions to the foregoing generalizations have been reported. Mazur and Bodansky (211) found that mouse brain hydrolyzed triacetin at a higher rate than ACh. This observation may have resulted from the employment of excessively high substrate concentrations. Several marine invertebrates were found by Augustinsson (9) to contain enzymes capable of splitting ACh at high rates and exhibiting little activity towards either acetyl- $\beta$ -methylcholine or benzoylcholine. He suggested that the ChEs from these sources might be more specific towards ACh than the enzymes previously studied. Hawkins and Mendel (146) reported that planaria and frog brain contain ChEs which are specific according to substrate criteria but which are extremely resistant to inhibition by physostigmine. Furthermore, the enzyme from planaria exhibited normal sensitivity to neostigmine but was not inhibited by high substrate concentrations.

The most satisfactory classification at the present time appears to be the one recently advanced by Nachmansohn and Augustinsson (235). They have applied the term "acetylcholine esterase" to the enzymes of nervous tissue and erythrocytes which catalyze the hydrolysis of ACh at optimal substrate concen-

trations at a greater velocity than any other known substrate, and which fit the general description previously given by Nachmansohn for "specific-ChE" Under the term "cholinesterase" they include both "acetylcholine esterase" and the various enzymes of sera and tissues which promote the hydrolysis of other choline esters, such as benzoylcholine and butyrylcholine, at relatively high rates (237, 264) Other hydrolytic enzymes which manifest specificity for such substrates as atropine (18) and monoacetyl morphine (89) are designated by the general term "esterase"

The pharmacological significance of the foregoing studies rests in the fact that endogenously liberated ACh, at the low concentrations in which it is present in the body, is apparently hydrolyzed exclusively by means of specific-ChE or acetylcholine esterase Consequently, anti-ChE drugs produce their typical cholinergic effects only when this type of enzyme has been inhibited beyond a certain threshold This fact was not fully appreciated prior to the studies of the alkyl phosphates, which were shown to be capable of producing practically complete inactivation of serum ChE without causing any significant symptomatology (211) Therefore, most of the earlier studies of the actions of anti-ChE drugs on the serum enzyme provide information only on such phases as absorption, excretion and enzyme kinetics, and do not supply any quantitative data relating enzyme inhibition to pharmacological responses

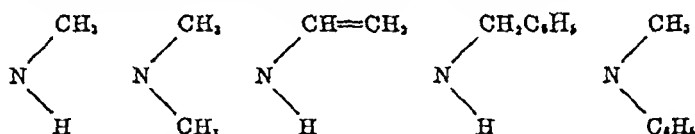
The distribution of the ChE enzymes throughout the animal kingdom and in the various tissues has been reviewed thoroughly in Augustinsson's recent monograph (10)

**STRUCTURE-ACTIVITY RELATIONSHIP** *Reversible inhibitors* The first drug known to inhibit ChE reversibly, physostigmine, has proved to be one of the most potent compounds possessing this action Early studies designed to elucidate the mechanism of its miotic action led to the synthesis of neostigmine another highly potent anti-ChE These investigations provide a typical example of the significant modifications of pharmacological activity produced by minor alterations of chemical structure, and reveal the difficulty met in attempting to draw generalizations relating these two factors

Most of the classical work of Stedman and collaborators and Aeschlimann and Reinert on the relationships between chemical structure and pharmacological activity of the homologues of physostigmine and related compounds was conducted before it was realized that these drugs are potent inhibitors of ChE After determining the structure of the physostigmine molecule and its inactive degradation product physostigmol (285), Stedman decided that the miotic action of the former was due to the urethane grouping He then prepared a series of urethanes from dimethylaminophenol (DMP) and hordenine ( $\text{HO}-\text{C}_6\text{H}_4-\text{p}-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_2$ ) and compared their miotic potencies in cats (282) All three isomers of the dimethylaminophenyl ester of methylcarbamic acid were found to be active, as well as the corresponding *m*-derivative of carbamic acid, the *o*-derivative of ethylcarbamic acid, and the methylcarbamide derivative of hordenine All phenylcarbamic acid esters were inactive When the tertiary basic group was converted to a quaternary ammonium group by the synthesis of the corresponding methiodide, a marked change occurred, the activity of the *m*-compounds was intensified, that of the *o*- and *p*-derivatives was abolished. This was interpreted, according to the polarity theory, as an indication that those compounds

which were derived from the most acidic phenol derivatives were the least active. It was noted that the active synthetic compounds and physostigmine have two structural features in common, both are substituted phenyl esters of methylcarbamic acid and both contain a basic group. Subsequently, a series of urethanes was synthesized from isomeric hydroxy benzyl dimethylamines (HBDM) and hydroxy-phenylethyl dimethylamines (HPEDM) and their miotic properties were investigated (283, 284). Here, too, the methylurethanes were found to be most active, the order of activity for the isomers of HBDM being  $c > p > m$ . Of the isomers of the methylurethane of HPEDM, the *m*-derivative showed extremely high potency which approached that of physostigmine, and it was given the name miotus. The methyl urethanes of choline iodide and tropeine were also tested and found to be inactive, thus confirming the view that miotic activity was associated only with phenyl esters. Following the announcement by Englehart and Loewi that eserine inhibits ChE (96), the above classes of urethanes were investigated from the standpoint of their ability to inhibit the hydrolysis of methyl butyrate and tributyrin (286). Whereas most of the miotics were highly potent in this respect, the two activities did not show close parallelism. Because of their assumed differences of penetrability *in vivo*, the authors did not consider this discrepancy to rule out the possibility of their producing miosis by esterase inhibition. They suggested that the mechanism of this inhibition probably depended upon the ester structures of the urethanes and their resistance to hydrolysis (287). A detailed pharmacological study was conducted with miotine, the three isomers of the methylcarbamic urethanes of HBDM, and physostigmine. All possessed similar pharmacological properties as revealed by their effects when administered alone or in conjunction with ACh, atropine, nicotine or curare. It was concluded that while the major part of the actions of physostigmine and miotine was dependent upon the inhibition of ChE, an additional factor of direct action on effector organs might be present (315).

Aeschlimann and Reinert (4) studied the structure-activity relationships of 45 additional urethanes. Because of the stability of the dialkyl and diaryl carbamic esters they concentrated on these types. Eserine like action was found to be the strongest in the carbamate esters of phenol bases with the following radicals attached to the carbamate nitrogen



The quaternary salts of the aromatic bases having the nitrogen attached directly to the benzene ring were more active than the hydrochlorides of the corresponding tertiary bases, whereas the reverse was true with compounds having the basic radical in a side chain. The quaternary salts in general were found to be relatively ineffective when administered orally. Two compounds, the dimethylcarbamic ester of 3-oxypheyltrimethylammonium methylsulfate (prostigmine or neostigmine) and the corresponding methylphenyl carbamate, were selected as promising drugs.

In addition to the above urethanes, several other quaternary ammonium compounds and tertiary amines are relatively potent inhibitors of ChE.

Methylene blue and other basic dyes having strongly dissociated quaternary ammonium groups are examples of the former type. Conversion of such dyes to tertiary leuco-bases by the addition of alkali results in the loss of their anti-ChE activity. Thionine, which differs from methylene blue in that the methyl radicals in the quaternary ammonium group are replaced by hydrogen atoms, is a considerably weaker inhibitor. This difference was attributed by Massart and Dufait (208) to the increased dissociation of the quaternary group accompanying substitution with alkyl radicals. They demonstrated a parallelism



between dissociation constant and anti-ChE potency with several such dyestuffs. When methylene blue is administered *in vivo*, however, it does not produce the same action as physostigmine (252), apparently because of two factors: the conversion of a large portion of the dye to the inactive leukobase at the pH of the blood, and its combination with the receptors of the effector cells to prevent the access of ACh (250). Although these authors found Congo red and other acid dyestuffs to be relatively inactive as ChE inhibitors, a recent report (166) has suggested that the anticholinergic action of Congo red may be partially due to an anti-ChE effect. The anti-ChE activity of crude curare preparations (213) has been shown to be due largely to impurities, since the purified alkaloids are very weak in this respect (135).

Among tertiary amines having anti-ChE activity, the toad-poison cinobufotenine flavinate, which bears a close structural resemblance to physostigmine, is fairly potent (277). Of several vesicants studied, Thompson (295) found  $\beta$ - $\beta'$  dichlorodiethyl N-methylamine and trichlorotriethyl amine to be moderately potent ChE-inhibitors *in vitro*, while carbomethoxy- $\beta$  chloroethyl nitrosamine caused greatest inhibition of the skin ChE when applied locally. The anti-malarials quinine and atabrine are potent inhibitors (308), whereas paludrine (21), like perazine and related compounds (322), shows little activity against brain ChE but inhibits serum ChE more strongly. Wright's studies of aromatic amino alcohols (319, 320) indicate that anti-ChE and antimalarial activities are unrelated.

There are numerous other anti-ChE drugs in which the amino nitrogen forms part of a heterocyclic ring. The Bernheims (17) noted that morphine and apomorphine in high dilution inhibit brain ChE, and suggested that some of their pharmacological actions might be due to this property. The imidazoline derivatives prisol, privine and otrivin exhibit weak anti-ChE activity but are relatively specific against the different types. Their inhibiting action against the amine oxidases is much greater (260). The action of strychnine (230, 231) has been attributed to its anti-ChE activity but its potency in this respect scarcely justifies such a conclusion in view of the small doses which elicit marked pharmacological effects. Inhibition of ChE is also produced by dilaudid, codeine, desomorphine (321), meperidine (28) and caffeine (242).

The purpose of the present review would not be served by listing all the drugs known to inhibit ChE. Such a table has been assembled by Augustinsson (10), and Bernheim (16) has reviewed the pharmacology of many of the compounds mentioned above.

As stated in the introduction, the fact that a compound is capable of inhibiting ChE does not imply that it produces its pharmacological effects by this mechanism. Moreover, a drug acting primarily as an anti-ChE at one site may act directly on the effector cells at another locus. This phenomenon is illustrated by the studies of Clark and Raventos (55) and Raventos (249, 250) on the pharmacology of some quaternary ammonium salts.

These authors investigated the interactions of homologues of the series  $(CH_3)_3NR$ ,  $R_4N$ ,  $R_nNR'_{(4-n)}$  and  $(CH_3)_3N(CH_2)_nCH_3$ , as well as their effects on the actions of ACh on frog and leech muscle, frog auricle and rat gut. Lower members of the series  $(CH_3)_3NR$  had only a curariform action on leech muscle, with the other test objects the lower members produced an ACh-like effect and summated with ACh. The higher members antagonized both the actions of ACh and those of lower members. The remaining series had comparable although somewhat more complex effects. Methylene blue, on the other hand, antagonized the action of ACh on the frog heart and rat gut, had a diaphasic effect on the frog rectus, and potentiated ACh action on leech muscle. On the last named test object, its anti-ChE action apparently predominated, whereas on the frog heart it acted as an antagonist in the same manner as the other quaternary ammonium salts studied.

It would seem likely, therefore, that many of the actions of the anti-ChE drugs, especially the less potent ones, may be due to similar direct effects. The response of a particular effector cell is dependent upon which type of action predominates at that site,—ChE-inhibition, direct effect or cholinergic blockade.

*Irreversible inhibitors* During the course of chemical warfare research it was established that the series of esters of fluorophosphoric acid inhibited ChE. Of the series, the di-isopropyl derivative was the most active. Also during the war years Bloch and Hottinger (22, 154) demonstrated that tri-orthocresyl phosphate was a potent anti-ChE (see below). At the close of the war the Technical Intelligence Committee investigating research progress in Germany learned that the Germans had been employing hexaethyltetraphosphate (HETP) as an insecticide. DuBois and Mangun (72) soon demonstrated that this alkyl phosphate was capable of inhibiting ACh-splitting enzymes. Although only few studies have been published on the structure-activity relationship of the alkyl phosphates relative to the inactivation of ChE, the outstanding contribution of Brauer (27) has provided much information in this field as well as basic facts pertaining to the reaction between enzyme and inhibitor (see below).

Brauer studied a group of 16 phosphate esters. He used as his source of enzyme the cholinesterase of fractionated human plasma (fraction IV-6). Highly active compounds were found among all types of phosphates including polyphosphates, phosphophosphines, thiophosphates and sulfonephosphates. Although the number of compounds studied was too small to permit detailed analysis of the effects of structure on the degree of anti-ChE activity, and the exact constitution of certain of the alkyl phosphates is not known, nevertheless Brauer was able to draw fundamental conclusions concerning the structure necessary for inhibition of ChE. Thus all the active compounds had in common the grouping  $P-O-R$  where R may be an alkyl or an aryl radical. However, the presence of this grouping alone is not a sufficient condition for activity. For example, in the following pairs of compounds the first member is inactive, the second active: (1) triethyl phosphate, tetraethylpyrophosphate, (2) trimethyl phosphate, dimethyl fluorophosphate, (3) tri-*p*-cresyl phosphate, tri-*o*-cresyl phosphate. Brauer points out that in each of the above pairs, the active compound contains an arrangement which would be expected to have a high free-energy content as follows: (1) the pyrophosphate linkage, (2) the analogous anhydride of hydrofluoric acid dimethyl phosphoric acid and (3) the steric strain which probably exists in tri-*o*-cresyl phosphate. Of the various compounds studied by Brauer, HETP and tetraethylpyrophosphate (TEPP) exhibited the highest activity and produced 50 per cent inhibition of the enzyme when employed in the molar concentrations of  $1.0 \times 10^{-9}$  and  $8.6 \times 10^{-10}$ , respectively.

*DYNAMICS IN VITRO Reversible inhibitors* In an earlier section it was pointed out that the hydrolysis of ACh by the non-specific enzymes of serum and various tissues is characterized by a direct relationship between reaction velocity and substrate concentration. This type of relationship is expressed mathematically by the well-known Michaelis-Menten formulation (222), according to

which a single molecule of substrate combines with one enzyme center to form a complex, the breakdown of the combination into the reaction products and the free enzyme proceeds as a monomolecular reaction. Other substances which can combine with the enzyme but which are not necessarily split by it act as inhibitors, the potencies of which are proportional to their affinities for the enzyme compared with that of the substrate. Matthes (209) employed these principles in one of the earliest quantitative studies of the hydrolysis of ACh by blood and serum and the inhibitory effect of physostigmine. He assumed that the inhibition was a non-competitive monomolecular reaction and demonstrated its reversibility by dialysis.

The inhibition of hydrolysis by an excess of substrate, as observed with the specific ChEs of nervous tissue and erythrocytes, represents a type of reaction that was studied by Haldane (134). In such cases, when reaction velocity is plotted against the logarithm of the substrate concentration, a bell-shaped curve is obtained, the peak of which occurs at the optimal substrate concentration. According to Haldane's interpretation, in order to bring about reaction, enzymes of this type and substrate molecules must combine at two spots, forming the complex  $E = S$ . In the presence of excess substrate, an inactive type of combi-

nation tends to occur,  $E \begin{array}{c} S \\ \diagup \\ \diagdown \\ S \end{array}$ , which competes with the formation of the reactive

complex. For specific ChE, it has been suggested that the two points of combination are represented by a group on the enzyme molecule which combines with the ester linkage of the substrate, and an appropriately located negatively charged group which combines with the positively charged quaternary nitrogen of ACh (9, 322). Combination between ACh and non-specific esterases, where the Michaelis-Menten formulation is applicable, is assumed to occur at only one locus (9). This concept also provides a possible explanation for the shifting of the optimal substrate level of specific ChEs by treatment with positively charged protamines, which presumably results in blocking of the negative groups of the enzyme molecules.

In an investigation of a group of enzymes which acted in accordance with the Haldane theory, Lineweaver and Burke (105) developed graphical procedures for determining the dissociation constants of the different complexes formed by the enzyme, and for distinguishing between competitive and non-competitive inhibition. This distinction was based on the assumption that with the former type the degree of inhibition is decreased with increasing concentrations of substrate in the presence of a constant amount of inhibitor, whereas when inhibition is non-competitive it is not influenced by substrate concentration. Applying this method to an investigation of the inhibition of a purified horse serum ChE by several compounds, Roepke (257) obtained results which indicated that inhibition was produced competitively by choline, carbaminoyl choline, arsenocholine, acetyl- $\beta$ -methylcholine, tetramethylammonium chloride

atropine and several other substances, but non-competitively by physostigmine and neostigmine. He noted, however, that in the presence of the last two drugs, rates of hydrolysis progressively increased during the determination periods, which indicated that equilibrium had not been attained between enzyme, inhibitor and substrate, and he suggested that this factor might have distorted his results. He also calculated the dissociation constants for the enzyme-inhibitor complexes by means of the Michaelis-Menten equations. Using modifications of the same types of mathematical treatments, Eadie (74) arrived at entirely different conclusions, namely, that the inhibition of dog serum ChE by physostigmine or neostigmine is competitive and that equilibrium is reached within a few minutes after mixing. On the assumption that two molecules of inhibitor combine with one of enzyme, he obtained extremely low dissociation constants.

A major departure from the foregoing methods of treating reversible enzyme-inhibitor combinations was made by Easson and Stedman (75) in a study of the kinetics of the system horse serum ChE-ACh-inhibitor (physostigmine or neostigmine). Instead of treating the reaction as a first order one, as previous workers had done on the assumption that the enzyme centers were present in infinitesimal concentration, they regarded it as truly bimolecular. This concept was greatly expanded and placed on a sound quantitative basis by Straus and Goldstein (293). They introduced the term "specific concentration," defined as the ratio of the molar concentration of enzyme or inhibitor to the dissociation constant of the complex formed. As determined by the *specific concentrations* of enzyme in a given system, three "zones of behavior" were described, characterized by significant differences in functions relating the concentrations of the components. In zone A, where the specific concentration of the enzyme is small and practically all the inhibitor exists in the free state, inhibition was shown to be a function of only the specific concentration of the inhibitor. In such cases, the classical equations assuming a pseudomonomolecular reaction were shown to be applicable. In zones B and C, however, where the specific concentrations of enzyme become increasingly greater, the reactions between enzyme and inhibitor must be treated as bimolecular and stoichiometric, respectively. Equational and graphical methods were presented for determining zone boundaries, approximate specific enzyme concentrations and the zone in which a given system is operating, and for treating reactions in each zone. The application of the method was demonstrated with the system horse serum ChE-physostigmine. The change in inhibition resulting from dilution of a system was stressed. Furthermore, it was suggested that at certain locations in the body where enzymes are highly concentrated, such as the neuromuscular junction, zone C relationships might operate. Consequently all potent reversible inhibitors would be approximately equally effective at such sites even though their individual potencies differed within wide limits. Goldstein (123) extended the study to include the competitive effect of the substrate in the system ChE-ACh-physostigmine. The same three zones of behavior were found to occur under this condition, and it was shown that the zonal phenomenon could be utilized for determining the number of molecules of substrate or inhibitor combining reversibly with a single enzyme.

center In contradiction to Eadie's conclusion, this number was found to be one in the system under study, and combination between ChE and physostigmine in moderate concentrations was shown to proceed slowly, as Roepke had observed In addition, methods were developed to correct for such factors as dilution, displacement of inhibitor by substrate and vice versa, and for determining the rate of destruction of the inhibitor These investigators have placed the study of reversible enzyme-inhibitor systems on a far more satisfactory quantitative basis, and have explained many of the earlier discrepancies mentioned above From a practical view point, most systems studied operate in zone A, and, as the authors point out, can be treated by the older methods provided the other factors mentioned are properly taken into consideration Thus, in Augustinsson's (10) recent investigations, in which he utilized only relatively dilute ChE systems *in vitro*, the equations of Michaelis and Menten, Haldane, and Lineweaver and Burke were found to be quite satisfactory for treating his data However, practically all *in vivo* studies of the degree of inhibition of a given enzyme produced by administration of a reversibly acting drug necessitate the application of Strauss and Goldstein's correction factors to avoid gross errors For the mathematical treatment of the above theories, reviews (10, 23) and the original papers should be consulted

Goldstein (124) has recently reported on the types of inhibition of a highly purified human plasma ChE produced by several compounds The group of reversible but non-competitive inhibitors included methylene blue, acriflavine, morphine, atropine, strychnine, amphetamine, phenobarbital, sulfanilamide, procaine, choline and acetyl- $\beta$ -methylcholine, all of which showed a 1:1 ratio for molecules of inhibitor to active centers Physostigmine, neostigmine and carbaminoylcholine inhibited reversibly and competitively As noted below, these three compounds were the only ones found by Koelle (169) to offer a high degree of protection for brain ChE against irreversible inactivation by DFP

Ellis and coworkers (90, 92, 93) have studied the kinetics of the destruction of physostigmine in some detail and have investigated the properties of the breakdown products The non-enzymatic decomposition of physostigmine in buffered solutions was found to be a bimolecular reaction, the rate of which was dependent upon the concentrations of physostigmine and  $\text{OH}^-$  In the presence of ChE, the velocity of destruction was also related to the concentration of the enzyme, provided it was not inhibited beyond about 80 per cent of its normal activity Physostigmine was converted to eseroline by hydrolytic cleavage of the carbamate group, and was then oxidized to the quinoid rubreserine This was converted to eserine blue, a compound of undetermined structure, which was further oxidized to eserine brown Rubreserine and eserine blue were shown to have strong anti-ChE activity (about 1/100th that of physostigmine) and showed comparable pharmacological effects, whereas eseroline and eserine brown were devoid of this property

*Irreversible inhibitors* It is obvious from the above discussion that the kinetics of the inhibition of ChE by reversible inhibitors are so complex, even under controlled conditions *in vitro*, that quantitative studies relating enzyme inhi-

bition to pharmacological actions *in vivo* are impractical if not impossible. The discovery of the irreversible anti-ChEs has provided the pharmacologist with a research tool that can be used much more effectively than the reversible inhibitors to elucidate the fundamental rôle of ACh and ChE in various physiological processes. The heuristic value of these drugs is evident in the extensive literature that has already accumulated. However, even in the case of irreversible anti-ChEs the interpretation of experiments *in vivo* must rest upon a fundamental understanding of the dynamics of the reactions between ChE and irreversible inhibitors *in vitro*, as well as a knowledge of the reactions of the inhibitors with substances other than ChE.

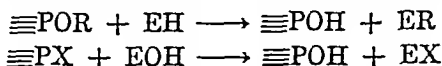
The irreversible anti-ChEs were first studied during the course of chemical warfare research. It was only at the termination of the war that security regulations permitted publication in the open literature. Consequently it is difficult to follow the chronological sequence of events in the development of this field from the open literature.

The first studies of the pharmacological actions of the alkyl phosphates apparently were conducted by Adrian, Feldberg and Kilby (3). They immediately deduced that the prolonged miosis that followed exposure to DFP could be explained more readily by ChE inhibition than by a direct action of DFP on effector cells. They then demonstrated the high activity of DFP in inhibiting ChE. Subsequently Mackworth and Webb (202) first suggested that the inactivation of ChE by DFP was irreversible since the activity of the enzyme could not be restored by dialysis. By an odd coincidence Bloch and Hottinger (22, 154) were conducting at the same time independent investigations on tri-*o*-cresyl phosphate. They observed that the compound irreversibly inhibits ChE and their papers provide the first published reports on ChE inhibitors of the alkyl phosphate type.

The extensive investigations of Mazur and Bodansky (211) further characterized the nature of the reaction between ChE and DFP. They demonstrated that the ChEs of different species and tissues varied in susceptibility to inhibition. In general, the enzyme in the sera of most species, the rabbit representing an exception, was more readily inhibited than that from red cells or nervous tissue. They were unable to reactivate the enzyme by dialysis, dilution or treatment with phosphatase, and concluded that the inhibition was irreversible.

More intimate details of the nature of the reaction between ChE and alkyl phosphate inhibitors have been provided by Brauer (27). He worked for the most part with HETP and TEPP, but his observations may apply to the entire group of alkyl phosphates which inhibit ChE. Brauer employed red blood cells and fractionated plasma as his source of enzymes. He observed that the relation between concentration of inhibitor and degree of enzyme inhibition was linear over a wide range. Such a stoichiometric relation between the number of active enzyme centers destroyed and the number of inhibitor molecules present would be anticipated in a reaction involving irreversible inhibition. Brauer further demonstrated that in the reaction between alkyl phosphate and ChE, the inhibitor as well as the enzyme is destroyed. Furthermore the resulting complex

formed contains no phosphorus Brauer has proposed the following reactions between enzyme and inhibitor which are compatible with his observations



where  $\equiv\text{POR}$  or  $\equiv\text{PX}$  is the inhibitor, EH or EOH the active enzyme, ER or EX the inactivated enzyme, R an alkyl or an aryl group, and X a halide

The alkyl phosphates show rather marked specificity in their chemical reactions when this is measured in terms of loss of anti-ChE activity (27) For example, anti-ChE activity is not lost in the presence of various amino acids, ethanol or phenol In fact ChE denatured with ethanol, acid or heat loses its ability to react with these inhibitors Crystalline human albumin and human fibrinogen also fail to react However, fractions of human plasma containing high globulin concentrations show some ability to inactivate TEPP, although less than the main esterase-bearing fractions

There is presumptive evidence that physostigmine, neostigmine and carbamoylcholine react reversibly with the same moiety of the ChE molecule as do DFP and TEPP irreversibly As will be discussed below, the prophylactic administration of certain reversible inhibitors of ChE protects animals from the effects of DFP (175) To explain this phenomenon Koelle (169) first reacted brain ChE with various reversible inhibitors, then added DFP and after a suitable interval dialyzed the mixture In the presence of any of the above three compounds, in contrast to numerous other inhibitors, the ChE was protected from inactivation by DFP The possible significance of this finding relative to competitive and non-competitive reversible inhibitors of ChE has already been discussed

Nachmansohn and coworkers have also studied the kinetics of the reaction between ChE and DFP (240, 241) They used as their enzyme a highly purified ChE obtained from the electric tissue of *Electrophorus electricus* as well as ChEs from other sources A stoichiometric basis of the reaction between ChE and inhibitor was observed The reaction between enzyme and DFP was found to depend upon the concentration of enzyme as well as that of inhibitor The greater the dilution of the enzyme, the higher is the excess of DFP required for inactivation so that at the low enzyme concentration generally used for manometric determination a ratio of 100,000 molecules of DFP to one of enzyme is required for 50 per cent inhibition This ratio is reduced to 25 to one at higher enzyme concentrations

Nachmansohn's group has presented evidence to show that whereas DFP inhibits cholinesterase immediately, irreversible inactivation is a function of time, temperature and concentration of inhibitor For a period of a few hours at low temperatures and low inhibitor concentrations, the enzyme-inhibitor complex can be largely dissociated with reactivation of the enzyme As the temperature and concentration of inhibitor are raised, this period becomes progressively shorter Nachmansohn postulates that a loose addition complex is first formed between enzyme and inhibitor, following which a chemical reaction which cannot

be easily reversed develops between the active group of the enzyme and the DFP molecule. The evidence which Nachmansohn and his associates marshal to support the theory of the rôle of ACh in axonal conduction (see below) depends almost entirely on the concept of an early reversible phase of the inactivation of ChE by DFP.

A discussion of the reactions of the alkyl phosphates *in vitro* would not be complete without mention of the susceptibility of the compounds to inactivation by hydrolysis. Most if not all members of the series are unstable in aqueous solution. However, their half-life is sufficiently long to permit most experimental procedures. Solutions in anhydrous oily solvents are stable. In fact a solution of DFP in peanut oil can be autoclaved or kept at room temperature for several months without loss of activity (170). Finally Mazur (210) has demonstrated that an enzyme present in liver and other tissues (phosphofluorase) is capable of inactivating DFP by breaking the bond between fluorine and phosphorus.

**REACTIONS IN VIVO** The failure of earlier workers to take into account the differences between the ChEs of the plasma and various tissues, or the kinetic factors involved in reversible inhibition resulted in conclusions which the data obtained did not vindicate. Thus, Manning, Lang and Hall (203) attributed a dual action to physostigmine,—ChE-inhibition with preservation of endogenous ACh and a direct stimulating action on effector cells,—because they were able to obtain responses by injecting additional doses of the drug after it had been given in amounts sufficient to produce maximal inhibition of serum ChE. Heymans *et al.* (149) more recently arrived at a similar conclusion regarding the actions of physostigmine, neostigmine and DFP, although they apparently did not determine directly the inhibition of tissue ChE. The quantitative studies of Clark and Raventos (56) on the kinetics of ACh-hydrolysis *in vivo* would be worth repeating in the light of the facts known at present.

Krayer, Goldstein and Plachte (176) investigated the inhibition of serum ChE and rate of disappearance of physostigmine from the bloodstream when the drug was given by single injections and continuous intravenous infusions to dogs. They applied the necessary correction factors for equilibrium, destruction of inhibitor, dilution and competition discussed above (123, 293). A given level of inhibition could be maintained indefinitely by intravenous infusion at a predetermined rate, when the infusion was stopped, serum ChE activity rose rapidly to normal regardless of how long it had been depressed. Fasciculation of skeletal muscles occurred when the level was below 15 per cent of normal. The rate of destruction of physostigmine by the organism, in which the kidneys appeared to play a small part, was found to increase with increasing concentrations of the drug, so that after the ChE activity of the serum was depressed below 30 per cent of normal, it was necessary to give increasingly larger amounts to produce further increments of inhibition. Similar results have been obtained by Root (258) with neostigmine in dogs and in patients with myasthenia gravis. Both the liver and kidneys of dogs were found to take part in the destruction of the inhibitor.



DuBois and associates (71) have demonstrated the importance of penetration in governing the site of action of an anti-ChE drug. Three compounds were found to cause approximately equal inhibition of rat brain and submaxillary ChE *in vitro* (carbamic acid, N,N-dimethyl-4 dimethylamino-3 isopropyl phenyl ester methiodide, physostigmine and neostigmine). When minimal lethal doses were injected subcutaneously, the inhibition of ChE from these two sources and from the serum showed marked differences.

DFP reacts with ChE *in vivo* to produce irreversible inactivation of the enzyme. The reaction presumably is similar to that which occurs *in vitro*. However, certain of the other alkyl phosphates apparently react somewhat differently *in vivo* than *in vitro* and these discrepancies will be discussed.

In early experiments in which human subjects were exposed to low concentrations of DFP, Mazur and Bodansky (211) observed that serum ChE was completely inactivated at a time when the subjects showed little or no response to the drug. This surprising finding was soon explained by studies which defined the relative susceptibility to inhibition by DFP of ChEs of different tissues. In general, it has been shown by several investigators that the acetylcholine esterase of nervous tissue and erythrocytes is less susceptible to inhibition by DFP both *in vitro* and *in vivo* than the ChE of plasma (2, 145, 147, 170).

DFP inactivates ChE *in vivo* with extreme rapidity. Thus death can occur within a few minutes after the intravenous injection of a dose capable of inactivating most of the tissue ChE. The signs and symptoms of acute and chronic poisoning are discussed below. The lethal dose of DFP in the monkey is approximately 0.2 mgm per kg. At the time of death the ChE activity of the brain is virtually zero (211). Assuming that DFP is distributed throughout the total body water, the concentration of anti-ChE in the body fluids at equilibrium would be approximately  $1.7 \times 10^{-6}$  M. The concentration of DFP required to produce 50 per cent inactivation of the ChE of monkey brain *in vitro* is  $3.2 \times 10^{-6}$  M. Moreover, the concentration of DFP required to inhibit horse serum ChE is the same for the native serum and a purified enzyme preparation from the same source. Likewise, if human brain extract is heated to destroy ChE activity, the addition of this extract to human serum does not affect the sensitivity of the serum ChE to inhibition by DFP. All these facts attest to the rather marked specificity of the reaction between DFP and ChE, both *in vivo* and *in vitro*. However, there is ample evidence that DFP can react both *in vivo* and *in vitro* with substances other than ChE. Evidence for a reaction between DFP and globulin was presented above. The lethal dose of DFP is much higher when the inhibitor is injected into the portal circulation than when introduced into the systemic circulation (150). This may be due to an enzymatic inactivation of the alkyl phosphate or possibly to a reaction between DFP and substances other than ChE. Also Harvey and coworkers (140) have shown that when DFP is injected into the brachial artery of human subjects and is excluded from the general circulation for a brief period by venous occlusion, no systemic effects of DFP are evident upon release of the occluding tourniquet. Inasmuch as the doses employed were large (2.0 mgm) it must be presumed that the major

portion of the DFP reacted with and was inactivated by substances other than cholinesterase. When neostigmine was administered in the same manner there was no evidence of localized destruction, and systemic as well as local effects were observed.

*In vitro* evidence that neostigmine and DFP react with the same moiety of the ChE molecule and that the presence of neostigmine protects the enzyme from irreversible inhibition by DFP has been presented above. The same antagonism can be demonstrated *in vivo* and has practical significance in therapy. Koster (175) first demonstrated that the prophylactic administration of physostigmine protected animals from the effects of DFP. Harvey and associates (140) showed in humans with myasthenia gravis that the injection of DFP into the brachial artery improved muscle strength in the treated arm for days. Neostigmine produced a similar response, but only for a few hours. If DFP was administered after neostigmine, the response in no way differed from that to neostigmine alone. Apparently the presence of neostigmine protects ChE from irreversible inactivation by DFP *in vivo* as well as *in vitro*. This finding is of great practical importance. In the treatment of glaucoma, myasthenia gravis and ileus, the alkyl phosphates are often employed only after the reversible ChE inhibitors have proved relatively ineffective. If an alkyl phosphate is to be given to a patient who has already received neostigmine or physostigmine, its administration should be delayed until the reversible inhibitor has been excreted.

The fact that DFP inhibits ChE irreversibly *in vivo* has provided the means for studying the rate of resynthesis of this important enzyme. Plasma cholinesterase apparently is formed in the liver and is resynthesized within a few weeks (130, 170, 211). The rate of resynthesis is appreciably diminished as a result of liver damage (130, 314). The ChE of brain and muscle is replaced much more slowly and as long as three months may be required after inactivation with DFP before normal ChE activity returns (170, 211). The curve depicting regeneration rates is parabolic, with 50 per cent recovery occurring within the first few weeks. Erythrocytes are apparently incapable of resynthesizing ChE and values for red cell ChE activity return to normal after the inactivated cells have been replaced in the course of their physiological destruction. Thus the rate of return of erythrocyte ChE activity provides information on the life cycle of the red cell (130, 170).

All the alkyl phosphates thus far studied irreversibly inactivate ChE *in vitro* and attempts to reactivate the enzyme have been uniformly unsuccessful. However the pharmacological responses which follow the administration of HETP or TEPP are much more evanescent than those elicited by DFP. For example, topical application of DFP to the eye produces a miosis which may last for days or weeks whereas the effect of HETP is gone within 12 to 24 hours (69). There is evidence of a preliminary nature that the regeneration of tissue ChE occurs much more rapidly following the administration of HETP or TEPP than following DFP, especially during the initial phase (69, 127). It has been postulated therefore, that HETP *in vivo* forms a more labile combination with ChE than does DFP, and a significant amount of ChE can be reactivated and thereby forestall pharmacological responses.

**AUTONOMIC EFFECTOR CELLS** The classical experiments of Loewi and his associates have resulted in the general acceptance of the hypothesis that stimulation of autonomic nerves results in the liberation of chemical substances at their endings, the so-called chemical mediators of the nerve impulse. The designation autonomic nerves as either cholinergic or adrenergic logically followed the demonstration of the nature of the chemical mediators. Further understanding of the mechanism by which stimulation of cholinergic nerves elicited discrete responses in effector cells was provided by studies of the properties and distribution of enzymes capable of hydrolyzing ACh. With this background, the classical observation of Englehart and Loewi (96) that physostigmine inhibits ChE affords an adequate explanation of the complex parasympathomimetic actions of the alkaloid and provides one of the rare instances in which the basic mechanism of action of a drug has been elucidated.

Studies of the effects of physostigmine on autonomic effector cells have proved invaluable in establishing present physiological concepts of autonomic function. Moreover the drug has been an essential tool in helping to establish firmly the incontrovertible rôle of ACh as the chemical mediator released from postganglionic cholinergic fibers. This has resulted in a large and familiar literature on the pharmacological actions of physostigmine and related compounds on autonomic effector cells which cannot profitably be reviewed at this time. Suffice it to say that there are few if any actions of physostigmine on smooth muscles and exocrine glands which cannot be explained on the basis of ChE inhibition and the consequent enhancement of the effects of endogenous ACh. Thus the response of an effector organ to physostigmine is largely conditioned by the activity of the cholinergic nerves which it receives. It also follows that physostigmine will exert no prominent actions on autonomic effector cells which have been deprived of cholinergic innervation.

Since the discovery of the irreversible anti-ChE activity of the alkyl phosphate compounds, these concepts have been confirmed and in no way basically altered. The actions of the alkyl phosphates on autonomic effector cells are those that would be anticipated of an irreversible inhibitor of ChE. The responses are similar to those obtained following the administration of physostigmine or neostigmine but they are much more prolonged. These will be briefly reviewed inasmuch as the literature is rather recent.

**Eye** The actions of the alkyl phosphates on the eye have been investigated more extensively than those on other organs (192, 268, 269, 306). Following exposure to the vapors of the alkyl phosphates or topical application of solutions, an intense miosis develops within a few minutes. This is followed shortly by spasm of accommodation with the lens fixed for near vision. Following a single instillation of DFP in normal human eyes the spasm of accommodation lasts for days and the miosis persists for weeks. On the other hand, the effects of HETP are much less persistent (69). The possible explanation of the evanescent action of HETP has been discussed above.

The mechanism of the miotic action of DFP has been investigated by Leopold and Comroe (192). They demonstrated that the chronically denervated pupil does not constrict following the topical application of DFP. Thus DFP, like

portion of the DFP reacted with and was inactivated by substances other than cholinesterase. When neostigmine was administered in the same manner there was no evidence of localized destruction, and systemic as well as local effects were observed.

*In vitro* evidence that neostigmine and DFP react with the same moiety of the ChE molecule and that the presence of neostigmine protects the enzyme from irreversible inhibition by DFP has been presented above. The same antagonism can be demonstrated *in vivo* and has practical significance in therapy. Koster (175) first demonstrated that the prophylactic administration of physostigmine protected animals from the effects of DFP. Harvey and associates (140) showed in humans with myasthenia gravis that the injection of DFP into the brachial artery improved muscle strength in the treated arm for days. Neostigmine produced a similar response, but only for a few hours. If DFP was administered after neostigmine, the response in no way differed from that to neostigmine alone. Apparently the presence of neostigmine protects ChE from irreversible inactivation by DFP *in vivo* as well as *in vitro*. This finding is of great practical importance. In the treatment of glaucoma, myasthenia gravis and ileus, the alkyl phosphates are often employed only after the reversible ChE inhibitors have proved relatively ineffective. If an alkyl phosphate is to be given to a patient who has already received neostigmine or physostigmine, its administration should be delayed until the reversible inhibitor has been excreted.

The fact that DFP inhibits ChE irreversibly *in vivo* has provided the means for studying the rate of resynthesis of this important enzyme. Plasma cholinesterase apparently is formed in the liver and is resynthesized within a few weeks (130, 170, 211). The rate of resynthesis is appreciably diminished as a result of liver damage (130, 314). The ChE of brain and muscle is replaced much more slowly and as long as three months may be required after inactivation with DFP before normal ChE activity returns (170, 211). The curve depicting regeneration rates is parabolic, with 50 per cent recovery occurring within the first few weeks. Erythrocytes are apparently incapable of resynthesizing ChE and values for red cell ChE activity return to normal after the inactivated cells have been replaced in the course of their physiological destruction. Thus the rate of return of erythrocyte ChE activity provides information on the life cycle of the red cell (130, 170).

All the alkyl phosphates thus far studied irreversibly inactivate ChE *in vitro* and attempts to reactivate the enzyme have been uniformly unsuccessful. However the pharmacological responses which follow the administration of HETP or TEPP are much more evanescent than those elicited by DFP. For example, topical application of DFP to the eye produces a miosis which may last for days or weeks whereas the effect of HETP is gone within 12 to 24 hours (69). There is evidence of a preliminary nature that the regeneration of tissue ChE occurs much more rapidly following the administration of HETP or TEPP than following DFP, especially during the initial phase (69, 127). It has been postulated therefore, that HETP *in vivo* forms a more labile combination with ChE than does DFP, and a significant amount of ChE can be reactivated and thereby forestall pharmacological responses.

of abdominal distention. However, they seriously interfere with other therapeutic applications of the drug in that patients may experience epigastric distress, abdominal cramps, nausea, vomiting and diarrhea. Atropine is of limited value in alleviating these symptoms.

**Cardiovascular system** The replacement of the pericardial fluid of the turtle heart with 0.0004 M DFP had no direct effect on the normal rhythm but greatly increased the degree of inhibition produced by vagal stimulation (58). In the isolated rabbit heart, large doses of DFP produced a brief period of inhibition, an action apparently unrelated to its anti-ChE effect. Subsequently, the preparation exhibited marked and irreversible sensitivity to inhibition by ACh (248). Neither therapeutic doses of the alkyl phosphates in humans nor small doses in animals have prominent effects on the cardiovascular system. Presumably the peripheral effects of ACh are balanced by nicotinic actions on the adrenal medulla and sympathetic ganglia. With large doses the blood pressure progressively falls to shock levels. Complete A-V block occurs. The cardiovascular effects can be prevented by atropine (69, 228). The actions of neostigmine on the cardiovascular system have been studied in detail by Mendez and Ravin (220), and its electrocardiographic effects have been analyzed by Goldfinger and Wosika (122).

**Miscellaneous** The effects of the alkyl phosphates on other autonomic effector cells have not been studied in detail but certain observations have been made in the course of studies of the general systemic actions of this group of anti-ChEs. DFP (26) and physostigmine (271) enhance secretion by the submaxillary gland. The sweat glands are stimulated by DFP. Thus 20 per cent of patients receiving DFP may perspire excessively (130). Less frequently lacrimation and salivation may be observed. However, increased secretion of these glands, especially the salivary, is prominent in animals receiving lethal doses. In the patients of Harvey and associates who received injections of DFP in the brachial artery, increased sweating occurred in the arm below the point of injection and persisted for days (140). The actions of DFP on the bladder are not prominent in patients receiving therapeutic doses. Urinary frequency is noted occasionally. Dogs receiving DFP over long periods exhibit urinary incontinence (171).

In summary, it may be said that nearly all the actions on the autonomic effector cells of the drugs discussed above can be accounted for by their inhibition of tissue ChE when they are given in moderate doses. High concentrations applied to isolated organs have many effects which are undoubtedly direct ones, but it is questionable that these are obtained *in vivo* following systemic administration.

**AUTONOMIC GANGLIA** The theory of the chemical mediation of the nerve impulse, first proposed to explain the transmission of impulses from postganglionic nerves to effector cells, was next extended to include synaptic transmission in autonomic ganglia. Thus, preganglionic fibers synapsing with either cholinergic or adrenergic postganglionic neurons were designated as cholinergic. The theory of chemical mediation at the ganglionic synapse has not received the general acceptance accorded the chemical transmission of postganglionic impulses.

physostigmine (7) and neostigmine (190), has no direct action on the sphincter muscle cells of the iris and exerts no pharmacological effect on the absence of a source of ACh

A decrease in intraocular tension accompanies the miotic action of DFP. In the rabbit this is invariably preceded by an initial rise in tension, a phenomenon which is occasionally seen in glaucomatous eyes. Von Sallmann and Dillon (306) have shown convincingly that the rise in tension results from the vasodilatation produced by the preserved ACh. As a result of arteriolar dilatation the choriocapillaries passively dilate and become more permeable to protein which gains access to the aqueous. The rise in tension can be prevented by vasoconstrictors.

The miosis and spasm of accommodation produced by DFP can be overcome by high concentrations of atropine. The weaker parasympatholytic agents and the sympathomimetic drugs are much less effective. Conversely, high concentrations of DFP (0.2 per cent) effectively antagonize an atropine-induced mydriasis.

Following the systemic administration of DFP the actions on the eye are not prominent. Therapeutic doses in humans cause few signs or symptoms referable to the eye, and miosis is prominent in experimental animals only when lethal doses are administered.

**Lung.** DFP, physostigmine and neostigmine cause constriction of bronchial muscle (126) and increased secretion of bronchial glands. These effects of the alkyl phosphates are very prominent in animals receiving large doses and contribute significantly to the lethal effects of this group of compounds. The actions on the lung are also apparent in humans breathing the vapors of alkyl phosphates. Following the systemic administration of therapeutic doses there may be a feeling of substernal tightness which may be due largely to cardiospasm.

**Gastrointestinal tract.** The isolated intestines of the rabbit and cat have been found to respond to DFP in the same manner as to physostigmine (11). After prolonged immersion in either drug the preparations continued to contract spontaneously but were not affected by further doses of the same or the other agent. Ingelfinger (160) has reviewed the earlier literature on the action of drugs on intestinal motility. The actions of DFP on the gastrointestinal tract *in vivo* are prominent. In animals there is an increase in tone of the intestinal muscle as well as in the rate and amplitude of contraction (228). In the human, the intramuscular injection of one to three mgm of DFP causes a marked increase in the motility of the small and large intestine (129). This overactivity subsides within three to six hours despite the fact that ChE presumably is irreversibly inhibited. However, the bowel remains hypersensitive to other stimulants such as morphine, posterior pituitary extract and neostigmine for as long as one to three weeks. For example, doses of neostigmine or pitressin which normally are without effect produce abdominal cramps, nausea and frequently vomiting and diarrhea in a DFP-sensitized individual. The intestinal actions of DFP can be antagonized by atropine and meperidine. Morphine also reduces the motility but increases the tone.

The actions of DFP on the bowel provide the basis for its use in the treatment

synaptic delays of some ganglionic cells, (2) the lack of correlation between synaptic delay and facilitation or inhibition, (3) the lack of correlation between after-potentials and responsiveness of ganglion cells and (4) repetitive responses to single nerve volleys. Physostigmine or neostigmine was found to slow the rate of decline of the ganglionic mediator, Eccles' negative results in this respect were attributed to his having used insufficient doses of physostigmine. In the light of his more recent findings, Eccles (79) has favored a dualistic theory of ganglionic transmission, with the major emphasis on the electrical component. Thus, he found the characteristic synaptic potentials set up by single or double preganglionic volleys to be unaltered by physostigmine, and considered that they resulted from the depolarization of the ganglion cell by the direct effect of the action currents of the preganglionic fibers. With tetanic stimulation of the preganglionic fibers, however, physostigmine decreased the stimulation frequency necessary to obtain summation and produced a prolongation of after-discharge associated with the appearance of a prolonged potential superimposed upon the normal synaptic potential. The prolonged potential was attributed to the effect of accumulated ACh which was presumably liberated normally in amounts too small to have any significant rôle in synaptic transmission. For further amplification of this viewpoint, the reader is referred to the recent reviews by Eccles (82, 84).

Arguments against the theory of the transmission of ganglionic impulses by ACh have also been advanced by Lorente de Nó (197, 198). When he repeated the perfusion experiments of Kibjakow (167) he obtained no direct correlation between preganglionic stimulation and ACh liberation, but noted that the latter was usually associated with damage to the cells, as revealed by histological examination. In keeping with these results were the findings of others that the amounts of ACh released from eserminized ganglia with natural circulation were extremely small (99, 200). It was suggested by MacIntosh that this might indicate a rapid resynthesis of ACh under physiological conditions to a precursor substance by some physostigmine-resistant mechanism (201). Recently, Emmelin and MacIntosh (95) have pointed out that the earlier perfusion studies were conducted with an eserminized Lock's solution of a high pH (about 8.5) which caused the tissues to become edematous within a short time. Using a modified perfusion fluid of pH 7.4, heparinized plasma or defibrinated blood containing adequate amounts of an anti-ChE agent (physostigmine, DFP or TEPP), they consistently obtained a constant amount of ACh in the venous effluent following each preganglionic volley.

Certain resemblances between the neuromuscular junction and autonomic ganglionic synapses have been noted (50). Just as motor denervation renders the former more sensitive to injected ACh, preganglionic section produces the same effect on the ganglion cells (259). Similarly, ACh and the anti-ChE drugs have a decurarizing action at both sites. Thus, Koppányi and co-workers (173) found that physostigmine antagonized the ganglionic paralysis produced by nicotine or curare. The same action was noted for both ACh and neostigmine by Cannon and Rosenblueth (50) and was attributed to the accumulation of sufficient ACh

to autonomic effector cells. However, it explains many of the actions of anti-ChEs at a ganglionic site. It is not within the scope of the present review to consider this controversial field in its entirety. Attention will be focused on those investigations which help to elucidate the pharmacological actions of the anti-ChE drugs on autonomic ganglia and on the contributions that these drugs have made to an understanding of the physiological events associated with ganglionic transmission.

In 1933, Kibjakow (167) reported that when the preganglionic fibers of the artificially perfused cervical ganglion of the cat were stimulated, the effluent contained a substance which when injected into the perfusate flowing to another ganglion resulted in contraction of the nictitating membrane. The substance was collected in eserinated perfusate and identified pharmacologically as ACh by Feldberg and Gaddum (99). They found that the addition of ACh to the inflowing perfusate elicited a similar action which could be potentiated by physostigmine. These results were interpreted to indicate that ACh normally acts as the transmitter of impulses from the preganglionic fibers to the ganglion cells. The theory was extended to include the transmission of splanchnic nerve impulses to the cells of the adrenal medulla by the confirmation of the earlier observation of Stewart and Rogoff (289) that the administration of physostigmine greatly augmented the output of epinephrine when the nerves to the gland were stimulated, and by the additional finding that ACh was liberated from the adrenals during such stimulation (100). Further studies by Feldberg and Vartiainen (101) showed that whereas small doses of physostigmine potentiated responses to preganglionic stimulation, larger doses had a paralytic effect, like that of nicotine, without interfering with the liberation of ACh. No ACh was liberated in the ganglion following antidromic stimulation of the postganglionic fibers. These results were confirmed by MacIntosh and coworkers (144, 165, 200) who also found that the inclusion of calcium, oxygen and glucose or some other metabolite in the perfusion fluid was necessary for the prolonged continuation of both the discharge of ACh and the transmission of the excitatory effect following preganglionic stimulation. The stimulating effects of potassium on the ganglion were attributed by Brown and Feldberg (36) to the liberation of ACh by the ion, while ganglionic inhibition by high concentrations of calcium was considered the result of its antagonizing this action of potassium.

The foregoing data were presented by their authors as additional support to the neurohumoral theory. However, Eccles (78) raised objections to the hypothesis on the basis of the extremely rapid decay of the synaptic transmitter (1.5–2.5 m sec) which he did not believe could result from the enzymatic hydrolysis of a chemical mediator. Furthermore, he had noted previously (77) that the administration of moderate doses of physostigmine had no effect on ganglionic potential waves or on the facilitation curve of the postganglionic fibers, large doses produced only reduction or abolition of ganglionic potentials. Rosenblueth and Simeone (261, 262) reinvestigated these aspects of the problem and concluded that the ACh theory provided a more satisfactory basis than the electrical theory for interpreting most of their observations, including (1) the long



erally be explained on the basis of relative penetration or some similar phenomenon. At the same time, other drugs with little or no anti-ChE activity, such as nicotine and pilocarpine, produce many of the same ganglionic effects, so that the possibility of a direct action on the ganglion cells cannot be excluded. It must be admitted that while the majority of the evidence at hand favors the theory, it remains to be proven. Bronk, Larrabee and coworkers (30, 185, 231), who have published numerous basic studies on the events concerned with ganglionic transmission, have pointed out that most of their findings can be interpreted by either the chemical or the electrical theory. The secondary rôle assigned to ACh in Eccles' dualistic theory of ganglionic transmission fits midway in his over-all hypothesis, according to which transmission at the neuromuscular junction is purely cholinergic and at central synapses purely electrical (84). Nachmansohn's theory (232) that transmission across the synapse is accomplished by the action current of the preganglionic fiber, with the subsequent liberation of ACh at a post-synaptic site, permits the same interpretation of the mechanism of drug action on ganglionic synaptic transmission as those postulating the release of ACh at the terminations of the preganglionic fibers.

**STRIATED MUSCLE** Shortly after Feldberg and Gaddum's (99) proposal of the neurohumoral hypothesis of ganglionic transmission, evidence of a similar nature was obtained by Dale, Feldberg and Vogt (67) for the transmission of impulses from motor nerves to striated muscle fibers. Although the subsequent developments of the chemical theory for impulse mediation at these two sites have followed somewhat parallel courses, neuromuscular transmission has received more extensive study than ganglionic. This has been due in part to the fact that the neuromuscular junction is more readily accessible to experimental investigation, either animal or clinical, and that it is possible to record directly both mechanical and electrical events following the transmission of impulses to muscle fibers. Furthermore, interest in neuromuscular transmission has been stimulated by the existence of a clinical entity, myasthenia gravis, where this process is defective and the defect can be more satisfactorily explained on a chemical than on a purely electrical basis. At the present time, the ACh theory is widely accepted for transmission at the neuromuscular junction, although there are several opponents to its application to ganglionic transmission.

Following the aforementioned experiments of Dale and coworkers (67), in which an ACh like substance was recovered from perfused striated muscle after indirect or direct stimulation, Brown, Dale and Feldberg (35), by the close intra arterial injection of  $2.0 \mu\text{gm}$  of ACh, produced contraction of the cat gastrocnemius equal in tension to that which followed maximal indirect stimulation. Chronically denervated muscle showed a response to as little as  $0.001 \mu\text{gm}$ . Small doses of physostigmine potentiated the response to ACh and converted the single twitch which normally followed a single maximal nerve volley to a brief tetanus. Curare or large doses of physostigmine or ACh produced inhibition of the response to ACh or nerve stimulation (229). Other ChE inhibitors, including methylurethanes of aromatic ammonium iodides and bordenine, were found to act qualitatively identically with physostigmine on mammalian muscle. Their potencies in this respect corresponded closely with their anti-ChE activities (12). Brown (32) recorded action potentials from whole muscles and single fibers, and found that the response to injected ACh was

to overcome the increased threshold produced by curare Both drugs were found to augment the effects of preganglionic stimulation when given in low doses or when endogenous ACh appeared to be present at the synapse in submaximal concentrations, when administered in high dose or just prior to a tetanizing preganglionic volley their actions were depressant Chou and deElío (53) confirmed the decurarizing action of physostigmine on the superior cervical ganglion, but under their experimental conditions obtained only a weak effect with physostigmine methiodide and none with neostigmine They ascribed these results to the failure of quaternary compounds to penetrate cell membranes adequately The same explanation might be offered for the findings of Schallek and Weirsmá (265) on the crayfish ganglion, where physostigmine and DFP produced a block, but ACh and neostigmine had no effect on synaptic transmission Neostigmine has also been shown to antagonize the depression of autonomic ganglia produced in dogs by dimethyl piperidines (305) and the tetraethyl ammonium ion (251)

Marrazzi and Jarvik observed that the application of DFP to the inferior mesenteric ganglion of the dog increased the number of post-synaptic fibers responding to submaximal preganglionic stimulation, without affecting the response of the non-synapsing fibers (206) Similar studies on arthropods have yielded less clear results In the sixth abdominal ganglion of the cockroach, Roeder and coworkers (256) found that DFP produced marked facilitation and after-discharge, alternating with periods of blocking, and caused sensitization to ACh However, neostigmine, physostigmine and strychnine produced only blocking They suggested that the anti-ChE compounds other than DFP might have a nicotinic paralytic effect on the ganglionic cells which prevented the development of facilitation, but concluded that in this species synaptic transmission is dependent upon ChE On the other hand, Bullock (42), investigating the properties of single synapses in the ganglion of the squid, found that DFP blocked transmission reversibly but only when applied in extremely high concentrations similar to those required for blocking axonal conduction No hyperexcitable phase was noted Studies on the crayfish ganglion have been noted above (265) It should be mentioned that the effects of various drugs differ markedly in these species at other sites, including the motor endplate (179-A) Koppányi and coworkers (174, 278) compared the effects of some alkyl phosphates and physostigmine on the pressor action of ACh in atropinized dogs, as a measure of their relative effects on sympathetic ganglia TEPP was the most active in this respect followed by HETP, eserine and DFP The curve relating dosage to pressor effect for each of these drugs, as well as for ACh and nicotine, showed a double peak The significance of the second phase of potentiation, at doses well above those producing inhibition, could not be explained from the data at hand

While practically all investigators believe that ACh plays a rôle in the metabolism of the autonomic ganglion and is in some way related to synaptic transmission, beyond this point agreement ceases Most of the pharmacological observations of the effects of anti-ChE drugs at this site can be interpreted on the basis of their acting only as enzyme inhibitors and with the full acceptance of ACh as the chemical mediator of transmission Exceptions to this statement can gen-

erally be explained on the basis of relative penetration or some similar phenomenon. At the same time, other drugs with little or no anti-ChE activity, such as nicotine and pilocarpine, produce many of the same ganglionic effects, so that the possibility of a direct action on the ganglion cells cannot be excluded. It must be admitted that while the majority of the evidence at hand favors the theory, it remains to be proven. Bronk, Larrabee and coworkers (30, 185, 231), who have published numerous basic studies on the events concerned with ganglionic transmission, have pointed out that most of their findings can be interpreted by either the chemical or the electrical theory. The secondary rôle assigned to ACh in Eccles' dualistic theory of ganglionic transmission fits midway in his over-all hypothesis, according to which transmission at the neuromuscular junction is purely cholinergic and at central synapses purely electrical (84). Nachmansohn's theory (232) that transmission across the synapse is accomplished by the action current of the preganglionic fiber, with the subsequent liberation of ACh at a post-synaptic site, permits the same interpretation of the mechanism of drug action on ganglionic synaptic transmission as those postulating the release of ACh at the terminations of the preganglionic fibers.

**STRIATED MUSCLE** Shortly after Feldberg and Gaddum's (99) proposal of the neurohumoral hypothesis of ganglionic transmission, evidence of a similar nature was obtained by Dale, Feldberg and Vogt (67) for the transmission of impulses from motor nerves to striated muscle fibers. Although the subsequent developments of the chemical theory for impulse mediation at these two sites have followed somewhat parallel courses, neuromuscular transmission has received more extensive study than ganglionic. This has been due in part to the fact that the neuromuscular junction is more readily accessible to experimental investigation, either animal or clinical, and that it is possible to record directly both mechanical and electrical events following the transmission of impulses to muscle fibers. Furthermore, interest in neuromuscular transmission has been stimulated by the existence of a clinical entity, myasthenia gravis, where this process is defective and the defect can be more satisfactorily explained on a chemical than on a purely electrical basis. At the present time, the ACh theory is widely accepted for transmission at the neuromuscular junction, although there are several opponents to its application to ganglionic transmission.

Following the aforementioned experiments of Dale and coworkers (67), in which an ACh-like substance was recovered from perfused striated muscle after indirect or direct stimulation, Brown, Dale and Feldberg (35), by the close intra-arterial injection of  $2.0 \mu\text{gm}$  of ACh, produced contraction of the cat gastrocnemius equal in tension to that which followed maximal indirect stimulation. Chronically denervated muscle showed a response to as little as  $0.001 \mu\text{gm}$ . Small doses of physostigmine potentiated the response to ACh and converted the single twitch which normally followed a single maximal nerve volley to a brief tetanus. Curare or large doses of physostigmine or ACh produced inhibition of the response to ACh or nerve stimulation (229). Other ChE inhibitors, including methylurethanes of aromatic ammonium iodides and hordenine, were found to act qualitatively identically with physostigmine on mammalian muscle. Their potencies in this respect corresponded closely with their anti-ChE activities (12). Brown (32) recorded action potentials from whole muscles and single fibers, and found that the response to injected ACh was

a short asynchronous tetanus, the frequencies of the components of which fell off along a characteristic curve. A qualitatively similar curve was obtained for the components of the tetanus following a single nerve volley to an eserinizied muscle, in which the initial intervals between spikes corresponded closely to the absolute refractory period. The effects of physostigmine were attributed to the maintenance in the muscle of a subliminal concentration of ACh. These studies were extended to denervated mammalian and amphibian muscle by Brown (33), and to avian muscle (37) and extrinsic ocular muscle (38) by Brown and Harvey. In the muscles of all these groups, the administration of physostigmine resulted in a response of contracture to ACh or to repetitive nerve stimulation, during which the initiation and propagation of excitation along the muscle fiber was blocked. This phenomenon was not observed in any other normally innervated mammalian muscle, but was considered analogous to the block of propagation obtained elsewhere with depressant concentrations of ACh, and presumably resulted from local depolarization of the fibers by the ester (38). Another effect of physostigmine noted in extrinsic ocular muscle was the apparent lowering of the threshold for direct electrical excitation during full curarization.

Harvey, Lilienthal and Talbot (141) have investigated the effects of rapid intra-arterial injection of ACh and neostigmine into the brachial artery of human subjects. Besides its vasomotor and sudorific effects, ACh produced severe pain, sensations of flexion and brief motor paresis. The injection of neostigmine resulted in more prolonged periods of localized motor paresis accompanied by visible muscular fasciculation. When similar injections were given to patients with myasthenia gravis, ACh produced a powerful localized muscular contraction, while neostigmine provoked an increase in motor power without the fasciculations or weakness noted in normal subjects (139). These and other observations (142) led the authors to postulate that the basic defect in myasthenia gravis is a reduction in the amount of transmitting agent released by nerve impulses, in association with an increased sensitivity of the muscle endplate, possibly resulting from a circulating toxic substance.

More recently, Harvey, Grob and coworkers (127, 140) have conducted similar experiments on the effects of the alkyl phosphates on neuromuscular conduction in normal human subjects and patients with myasthenia gravis. The compounds were injected into the brachial artery and the drug was kept localized by a brief period of venous occlusion. In this manner they were able to obtain maximal effects in the muscles of the forearm with a minimum of systemic side-actions. Following the administration of DFP to normal individuals there was no change in the voltage of the muscle potential in response to a single maximal motor nerve stimulus. However, the response became repetitive in nature and the initial spike was followed by a series of smaller potentials which showed progressive decline in voltage. When two maximal nerve stimuli were delivered, the voltage of the second muscle action potential was reduced. When the nerve was stimulated repetitively, the second response was greatly depressed but the third and fourth showed a remarkable recovery. This last observation is in distinction to the effects of neostigmine, where the muscle action potentials following repetitive nerve stimulation showed a progressive decline. The authors could offer no explanation for this discrepancy. The maximal effects of DFP on muscle action potentials developed within 30 to 60 minutes and were apparent for weeks. In contrast, those of neostigmine given in a similar manner developed within five minutes and were gone within an hour.

The electromyographic effects of TEPP (127) following intra-arterial injection in normal and myasthenic subjects were described as being intermediate between those of neostigmine and DFP in respect to duration of action and degree of localization. In normal subjects, TEPP produced a progressive decline in the height of the muscle action potential evoked by a train of motor nerve stimuli, similar to the picture seen after neostigmine. In other respects, the actions of the three compounds were qualitatively identical.

The intra-arterial injection of curare antagonized completely the effects of DFP on the electromyogram. Thus when the effects of the two drugs neutralized each other the muscle action potentials were of normal voltage and there were no repetitive responses (140).

The contrasting effects of DFP on muscle function in the normal and myasthenic individual should be emphasized. The normal subjects in the study of Harvey and coworkers developed fasciculation and motor weakness following the intra-arterial injection of DFP, the degree and duration of which was proportional to the dose employed. Following large amounts (20 mgm), pronounced paresis occurred and strength returned slowly over a period of 11 weeks. The effects of DFP on neuromuscular function in the patient with myasthenia gravis were entirely different. Before the administration of an anti-ChE the electromyogram of a myasthenic individual resembles that produced by curare. A typical depression of the second of two action potentials occurs in response to a pair of maximal motor nerve stimuli. When a train of stimuli are applied to the nerve, there is a progressive decrease in the size of the muscle action potentials. The administration of DFP corrected the abnormal electromyogram of a patient with myasthenia gravis so that the response to paired or a train of stimuli was the same as that of a normal individual without DFP. The effects of the anti-ChE were apparent within 15 minutes after intra-arterial injection and were maintained for days. No muscular fasciculation occurred and muscle strength was greatly increased (140).

The sensitization of the motor endplate to ACh in myasthenia noted by Harvey's group (194) has been denied by Acheson and associates (1) who gave intra-arterial injections of ACh in much smaller doses to myasthenic and normal subjects. Although the threshold doses for producing contraction varied greatly, they found no significant difference between the two groups with respect to either the range of threshold doses or the type of contraction. Harvey and coworkers (194) have confirmed this more recent observation. Inasmuch as fasciculation is dependent upon the synchronous firing of entire motor units (70) it was suggested that its appearance in the above studies might have been due to stimulation of some portion of the motor nerve fiber by ACh. This is in keeping with the explanation offered by Masland and Wigton, who found that the fasciculations resulting from the intra-arterial injection of large doses of ACh into the leg muscles of cats were accompanied by centripetally conducted spike potentials in the corresponding motor nerves (207). On the other hand, Eccles and coworkers (86) have demonstrated that retrograde transmission from endplate to motor nerve can occur in eserinated preparations immediately after conditioning volleys have passed through the nerve in the normal direction. Axon reflexes in-

volving entire motor units, initiated by such retrograde impulses, could also explain the fasciculation observed with massive intra-arterial doses of ACh. As would be expected, chronically denervated muscle does not exhibit fasciculation following the administration of physostigmine (182). The increase in fibrillation produced by anti-ChE drugs in such preparations (260, 223) can hardly be attributed to any specific mechanism, since the same effect results from numerous types of stimuli.

The studies of Harvey and associates on the effects of DFP and TEPP on muscle function recall to mind the syndrome of "ginger-paralysis" which was prevalent in the United States during the days of prohibition. Field investigations of the U. S. Public Health Service revealed that the paralysis occurred in individuals who drank certain lots of extract of Jamaica ginger, and M. I. Smith and coworkers (275) soon identified the offending agent as tri-orthocresyl phosphate. At that time the mechanism of action of the alkyl phosphates was entirely unknown. Now, however, the investigations of Hottinger and Block (154) have established the fact that tri-orthocresyl phosphate is an irreversible anti-ChE. Toxicity studies in dogs, cats and rabbits attest the ability of DFP to cause marked muscular paralysis and Harvey and associates have shown that a single large dose of DFP in humans can cause a paresis of long duration in normal muscle. It is possible, therefore, that the syndrome of "ginger-paralysis" is a reflection of the effects of the prolonged loss of ChE at the neuromuscular junction.

DFP has been found to act qualitatively identically with physostigmine at the neuromuscular junction of the cat (34, 157). However, when administered other than intra-arterially, relatively large doses were required to produce its effects at this site, due to the great uptake of the drug by the ChE of the plasma and other tissues. This would account for the fact that it does not appear to produce as marked fasciculations and muscular twitchings as does physostigmine after systemic administration (3).

In cats chronically poisoned with DFP, Hunt and Riker (158) observed effects similar to those seen in dogs (171), consisting of ataxia, muscular weakness and fasciculations. The altered response to intra-arterial ACh in these animals resembled that obtained after chronic denervation, being characterized by increased sensitivity to ACh and a prolongation of contraction, also, tetanus was not maintained with faradic stimulation of the nerve. Coppée and Bacq (61) found no differences between the actions of physostigmine and DFP on the isolated nerve-muscle preparation of the frog other than the failure of DFP to reinforce contractions with indirect stimulation, an effect observed with difficulty in this species after physostigmine (35, 151). Finerty (105) reported that the DFP potentiation of the effects of ACh on the frog rectus abdominus was much more marked in unbuffered Ringer-Locke solution, where the pH was 3.3, than at pH 7.0. Several factors, including the effect of pH on the rate of hydrolysis of DFP, might have played a part in producing this effect.

Guyton and MacDonald (132) made the interesting observation that the intra-arterial injection of ACh or nicotine, but not of neostigmine, produced muscular contractions in guinea pigs poisoned with botulinus toxin. This fact and the

histological appearance of the motor endplates suggested that the toxin acted by interfering with the production or liberation of ACh. The spasticity produced in the rat gastrocnemius by the local injection of tetanus toxin was found to be increased by DFP but not modified by curare or neostigmine (133).

Feng *et al* (102, 103) and Eccles and associates (85, 86) have utilized a more basic approach to the problem of neuromuscular transmission by recording endplate potentials (e.p.p.'s) of muscle fibers stimulated via the motor nerves under various conditions. The most recent findings of the latter group have been summarized in a review (84) in which, contrary to his earlier opinion (80, 82), Eccles concludes that the transmission process at this site is purely cholinergic. Eccles, Katz and Kuffler (86) had found that physostigmine produced an increase and lengthening of the e.p.p. in curarized muscle, and with repetitive stimulation brought out a slow wave of potential at the endplate which was scarcely detectable in the absence of the drug. The endplate potential was shown to be responsible for initiating the muscle impulse (178). Although these findings were originally interpreted as being in full accord with the neurohumoral theory, further studies by Fillenz and Hanafin (104) emphasized that the slow wave was much more markedly affected by physostigmine than was the initial brief phase. This led to the adoption of a dual theory in which the slow phase was attributed to ACh, the brief phase to the setting up of catelectronic polarization by the direct effect of the action current of the presynaptic axon. A similar conclusion was arrived at by Coppée (60) as a result of his studies of transmission in the frog sartorius. However, Eccles and MacFarlane (87) have recently found both phases to react identically by several tests (curarine and ACh effects, temperature coefficients, potentiation and decline with repetitive stimulation), so that the brief phase, by analogy, is now considered by Eccles to result from the action of ACh.

Evidence suggesting that neostigmine has a direct action on the neuromuscular junction has been published by Riker and Wescoe (254). After completely inactivating the ChE of the cat gastrocnemius by the intra-arterial injection of DFP, they obtained typical normal contractions following intra-arterial injections of ACh or neostigmine. Harvey and coworkers (194) were unable to confirm this observation but do not consider their negative result conclusive. Mequel (226) reported that the complete inactivation of the ChE of the frog rectus abdominus did not prevent its being further sensitized to ACh by physostigmine or neostigmine, and concluded that both compounds acted directly on the muscle. Bacq (11) obtained a similar result with the toad but not with the frog rectus.

As Riker and Wescoe have pointed out, the chemical and pharmacological similarities between ACh, carbaminoylecholine and neostigmine point strongly to the likelihood that the last-named compound exerts a direct effect on skeletal muscle. However, it does not necessarily follow that this is its primary effect following the systemic administration of small doses. That neostigmine does not act directly on the unstriated muscle of the iris sphincter has been mentioned (7).

The narrow dosage range in which TEPP is therapeutically effective and non-toxic in myasthenia gravis patients, compared with the much wider dosage range

possible with neostigmine, led Grob (127) to suggest that the latter drug may act by some other mechanism in addition to its anti-ChE effect. All his results with TEPP were explainable on the basis of ChE inhibition. Beck and Frommel (13) decided that neostigmine acts directly on skeletal muscle from their measurements of its lowering of the tetanizing frequency for human and guinea pig muscle, although they secured no direct evidence for such a mechanism.

An entirely different mode of action of neostigmine has been claimed by two groups. The muscle potassium content in myasthenia gravis patients, but not in normal subjects, was reported by Cummings (65, 66) to fall concomitantly with a rise in serum potassium following the injection of neostigmine. Thompson and Tice (296) obtained the opposite effects in dogs and rats, while their results with myasthenic individuals were inconsistent. However, both groups of investigators attributed the beneficial action of neostigmine in myasthenia to its effects on potassium balance rather than to its anti-ChE activity. It is quite possible that these effects were the result of epinephrine liberation (100), since the hormone has been reported to produce either a rise or fall in serum potassium depending upon the conditions of the experiment. No significant or consistent changes in serum potassium were noted in dogs following sufficient doses of DFP to produce severe nicotinic effects (172).

*Curare antagonism.* The long-established antagonism between physostigmine and curare and the similarity between the signs of curare poisoning and the weakness in myasthenia gravis led to the original trial of physostigmine (309) and later of neostigmine (310) in the treatment of this disease. Early quantitative studies of this antagonism by Briscoe (29) and Cowan (62, 63), and the more recent work of Bulbring (40, 41) with the isolated phrenic nerve-diaphragm preparation, have indicated that curare produces its effect, in broad terms, by raising the threshold for ACh, and that the decurarizing action of physostigmine and related drugs is directly associated with their anti-ChE activity. However, Huidobro and Jordan (156) obtained a very similar decurarizing effect with nikethamide, a compound which has no anti-ChE activity. Huidobro's (155) studies of the effects of several other compounds on the curare-neostigmine antagonism led him to conclude that this phenomenon could not be explained adequately by the threshold hypothesis. The failure of Unna and Kimura (300) to obtain significant protections against *d*-tubocurarine poisoning in mice can be attributed to the previously mentioned observations (34, 157) that DFP localizes poorly at the neuromuscular junction following systemic administration. In rabbits, Chase and coworkers (52) found DFP to afford a prolonged definite increase in the "head-drop" dose of *d*-tubocurarine.

The studies of Eccles and associates, which have focused attention on the end-plate potential as the critical stage in neuromuscular transmission, have provided more direct information on the mechanism of curariform action. Progressive curarization was found to diminish gradually the e.p.p. following nerve stimulation until, at about one-third of its normal height, it failed to initiate the muscle impulse (178). The reasonable suggestion was advanced that curarine opposes the depolarizing action of ACh by combining with the same chemical receptors



at the endplate region (86) The additional presence of moderate concentrations of an anti-ChE drug would thus favor the transmitter action of ACh by preserving temporarily greater amounts to compete with curarine for the receptors Whereas moderate concentrations of physostigmine alone were shown to increase the e.p.p., excessive amounts caused it to become decreased and further prolonged The authors were inclined to attribute this effect to a combination between physostigmine and the receptors, similar to the postulated action to curarine However, it is possible that this picture might have been produced by a persistent depolarizing action of excessive endogenous ACh, since prolonged depolarization is also known to block transmission Furthermore, when used in high concentrations, numerous unrelated anti-ChEs (177), as well as ACh itself (229), are "curariform" with respect to their effect on the mechanical response of muscle Measurements of the demarcation potentials produced by various anti-ChEs at the endplate under appropriate conditions, similar to those made by Kuffler (179) with ACh and other drugs, should settle this point These and related studies have been discussed in detail by Kuffler (179-A)

Erythroidine (276) and quinine (136) appear to be antagonized by neostigmine in the same manner as is curare Procaine, in addition to possessing a curariform action, interferes with the liberation of ACh at the neuromuscular junction (137, 161)

The effects of curare, ACh and other drugs have been studied by means of micro-application techniques by Buchthal and Lindhard (39) and histologically by Carey (51) Reinvestigation of certain phases of their work in the light of more recent developments might prove fruitful

The reviewers are in accord with the theory that synaptic transmission between motor nerve endings and the motor endplate of striated muscle is chemically mediated Certainly, the actions of the anti-ChE drugs at the neuromuscular junction can most readily be explained on the basis of such a theory

**CENTRAL NERVOUS SYSTEM** The comprehensive review by Feldberg (98) which appeared in 1945 contained an extensive coverage of the literature on the actions of anti-ChE agents at central sites Consequently, the present discussion will be concerned chiefly with publications that have appeared since then Feldberg stated "The present position of the theory of acetylcholine as central transmitter is all but settled" While admitting that certain facts were difficult to reconcile with the theory, he mentioned as supporting lines of evidence the presence of ACh and ChE in the central nervous system, the ability of nervous tissue to synthesize ACh, and the central effects of ACh and the anti-ChE agents Most of the subsequent investigations have been along the same lines One important new research tool has appeared, however, in the form of the alkyl phosphates Results already obtained with these irreversible anti-ChE agents suggest that they will add considerably to the understanding of central nervous mechanisms

Since Sjostrand (272) first reported on the electroencephalographic effects of applying ACh and related drugs to the cerebral cortex, several investigators have

employed this technic. The significance of such studies has been questioned by Eccles (83) on the basis of the high concentrations necessary to evoke effects and the osmotic factors involved. However, Miller (224) obtained excitation of the hypoglossal nucleus of the cat by applying bits of test paper soaked in 1:50,000,000 ACh to the floor of the fourth ventricle. The action was potentiated by physostigmine and abolished by atropine. Merritt and Brenner (221) noted that the cortical application of relatively low concentrations of ACh (0.05–0.5 per cent) plus neostigmine, or of more concentrated solutions of ACh alone, resulted in an EEG pattern in the cat which resembled that of grand mal. The effect was blocked by the subsequent application of diphenylhydantoin. The cortical suppressor areas have likewise been shown to be sensitive to the application of ACh and eserine by Becket and Gellhorn (14). The effect was manifested by a temporary diminution of electrical activity in the corresponding motor areas of the cortex, and by the failure of the latter to respond to direct electrical excitation, as indicated by electromyograms taken from the represented muscles. The authors also noted that the direct application of a purified preparation of ChE from dog pancreas to the cortex resulted in a diminution of electrical activity following afferent stimuli or the application of convulsant drugs. This latter finding is reminiscent of Mendel and Hawkins' (215) observation that the intravenous administration of a ChE preparation abolished the pupillary reflex in rats. Forster and associates (109, 110) have described a depression of cortical electrical activity immediately following the application of ACh, which was succeeded by the appearance of ACh discharges. The depression was accompanied by decreased cortical response to various sensory stimuli, and spread over the cortex in a linear fashion. It also appeared in distant areas in which subsequent ACh discharges were not apparent. The primary type of ACh discharges remained sharply localized, while secondary and tertiary discharges spread along what appeared to be neuronal paths. The significance of these observations remains open to speculation, relatively high concentrations of ACh were used.

A study by Bornstein (24) indicates that the liberation of ACh may be partially responsible for the syndrome immediately following concussion. When dogs and cats were subjected to experimental concussion under light anesthesia, the cerebrospinal fluid was found to contain abnormal amounts of ACh for as long as 48 hours afterwards. During this time characteristic behavior and EEG patterns were noted, both of which were abolished by atropine. The administration of equivalent concentrations of ACh by perfusion over the exposed cortex or by intracisternal injection resulted in similar changes which atropine likewise corrected.

Emmelin and Jacobsohn (94) have developed a technic for introducing drugs into the hypothalamic region of cats, in which the material remains confined to the limits of the third ventricle. ACh, physostigmine or neostigmine given in this manner in doses of 50  $\mu$ gms produced respiratory and sympathetic effects (apnea, inhibition of motility and tone of gut and bladder, decreased volume and acidity of gastric juice, increased volume of salivary secretion) similar

to those obtained by electrical stimulation of definite hypothalamic regions. Removal of the celiac ganglion and adrenals prevented the response of the intestine but not that of the bladder, confirming the inference that the drugs acted on sympathetic centers. Similar effects were observed in human subjects by Henderson and Wilson (148) following the injection of ACh and physostigmine into the lateral ventricles. Direct evidence that ACh stimulates the cells of the supraoptic nucleus and thus causes secretion of the antidiuretic hormone has been obtained by Pickford (245). ACh (7  $\mu$ gms) or physostigmine (8  $\mu$ gms) injected into this region in dogs produced inhibition of urinary flow which was not obtained with injections into nearby areas or after hypophysectomy. Sensitization to ACh following denervation, which Brown and coworkers (35) demonstrated in striated muscle, occurs also in the central nervous system. Stavraky and associates (280) removed portions of a frontal lobe in cats and observed that intravenous injections of ACh then resulted in striking motor and sympathetic manifestations on the contralateral side. These effects were presumably initiated in neurones which previously had been connected with the ablated areas. When patients with lesions of the premotor and motor cortices were given intravenous injections of acetyl- $\beta$ -methylcholine, similar effects were noted (107). Deafferentation of the limbs of cats sensitized the motor neurones of the anterior horns to both nervous and chemical stimulation. The chemical sensitization was not specific for ACh, however, and held for a variety of stimulants (281, 294).

Calma and Wright (48) have recently summarized the findings of several investigators concerning the central effects of physostigmine on reflexes. Potentiation occurred most frequently, although transient or predominant inhibition was not uncommon, depending upon the technique and preparations employed. In comparing intrathecal with intravenous administration in the cat, they found that the concentration in the spinal fluid had to be brought to approximately 200 times that required in the blood to produce certain reflex effects, indicating that physostigmine penetrates the white matter poorly. With the former route they obtained increase in the knee jerk, crossed extensor reflex, and jar reflex, along with an increase and prolongation of after-discharge. Effects on the flexor reflex were inconstant. Physostigmine also gave evidence of affecting irradiation, occlusion and facilitation. The last mentioned phenomenon appeared to be dependent on some degree of "long circuiting", contrary to the finding of Wikler (316) that in the cat, physostigmine produced enhancement of 2-neuron arc discharges but had little effect on multineuron arc discharges. In an earlier publication, Schweitzer, Stedman and Wright (270) obtained a close parallelism between the anti-ChE activities of a number of physostigmine-like compounds and their effects on spinal reflexes. However, most of the tertiary ammonium bases were excitatory, whereas the quaternary compounds were inhibitory in their central actions. The difference was attributed to the ability of the free bases to penetrate the cell membranes and inhibit intracellular ChE.

From studies of synaptic potentials in the anterior horn cells of the cat and frog, Eccles (81) has concluded that ACh plays no significant rôle in transmission

in monosynaptic pathways in the spinal cord. Soaking the frog or cat cord in physostigmine (1:10,000) did not modify the cataleptic potentials set up in the motoneurons by afferent impulses at frequencies of 210 to 400 per minute. Similar treatment with neostigmine and ACh was likewise without effect (83). The author believes that the synaptic potential, which is considered homologous with the endplate potential in striated muscle, is produced solely by the direct electrical effect of the action currents of the dorsal root fibers. However, these studies do not provide direct evidence of the degree of inactivation of ChE in the regions of the synapses. As mentioned above, penetration of the spinal cord by physostigmine is apparently limited (270), the quaternary compounds are probably even less permeative. It has been shown with DFP that the ChE in the central nervous system must be inhibited to a considerable extent before effects are apparent (170, 211, 236).

Gesell and associates (106, 115, 116) have advanced the theory, backed by considerable experimental work, that central and peripheral nervous integration is accomplished largely by the inhibiting effect of acid on ChE. The chief source of the acid is considered to be  $\text{CO}_2$ , which because of its high diffusibility is held to produce rapid intraneuronal changes in pH as a reflection of its concentration in the blood. Unanswered is whether under conditions of moderate hypercapnia the intracellular pH can fall to sufficiently low values to produce significant inhibition of the enzyme. The hyperpnea produced by  $\text{CO}_2$ , like that following DFP, physostigmine or neostigmine, was found to be antagonized by atropine (112). A supplemental relationship between ACh and  $\text{CO}_2$  in the regulation of cerebral circulation has been suggested by Darrow and coworkers (68).

Slaughter and associates (273, 274) obtained marked potentiation of the analgesic effects of morphine and other opiates with small doses of neostigmine. This action was not found by Andrews (8), most of whose subjects were addicts, but was confirmed by Flodmark and Wramner (108). The latter group noted that physostigmine or neostigmine given alone also produced elevation of the pain threshold. Wramner (318) has found that the same potentiation exists in the Straub test for morphine in mice. It should be emphasized that the potentiation noted in these studies does not indicate a common mode of action of these two drugs. The effect is equally well explained by the assumption that morphine has a direct ACh-like action on the neurones involved, while neostigmine or physostigmine acts primarily by preserving endogenous ACh.

The alkyl phosphates as a group exhibit more prominent central actions than do the reversible anti-ChEs. This is probably due to the high lipid-solubility of the alkyl phosphates and the rapidity with which they gain access to nervous tissue and reduce the ChE activity of the brain to critical levels. Grob (127) found that the oil-water partition coefficient of DFP, which has marked central effects, was considerably higher than those of neostigmine or TEPP, the central actions of which are much less apparent. Many members of the series are potent convulsants and animals apparently die of central rather than peripheral actions. The convulsions are clonic and tonic and continue without interruption. It is of extreme interest that they can be rapidly and completely abolished

by low doses of atropine (117) In curarized cats and monkeys the administration of DFP produces changes in the EEG characterized by an increase in frequency of discharge and a decrease in voltage Again these effects can be rapidly abolished by atropine or scopolamine (313)

DFP also has marked central effects in humans and these greatly detract from the therapeutic usefulness of the drug (130) Normal human subjects receiving full therapeutic doses of DFP over protracted periods develop the following symptoms referable to the central nervous system, listed in order of frequency excessive dreaming, insomnia, jitteriness and restlessness, increased tension, emotional lability, subjective tremulousness, nightmares, headache, increased libido, giddiness, drowsiness, paresthesias, mental confusion, visual hallucinations and tremor Changes in the EEG occur and consist of greater variations in potential, increased frequency (with increased beta rhythm), more irregularities in rhythm and the intermittent appearance of abnormal waves similar to those seen in patients with grand mal epilepsy The increased electrical activity of the brain can be inhibited immediately by the administration of atropine The prophylactic administration of atropine can delay the appearance of EEG changes for several weeks However, symptoms of central origin can be prevented for only a few days and appear in the absence of abnormal electrical activity of the brain (128)

DFP also increases spinal cord activity in humans (130) This was particularly evident in two patients with late central nervous system syphilis who had upper motor neurone lesions These patients had spastic paraplegia of the lower extremities and no disturbance in the innervation of the upper extremities In both, the administration of DFP resulted in intermittent and involuntary spontaneous movements of the thigh and calf muscles which were increased greatly by passive stretching of the muscles and by voluntary movement Presumably the upper motor neurone lesions sensitized the anterior horn cells to stimulation by ACh, these patients are the clinical counterpart of the experimentally denervated animals of Cannon and Hamovic (49-A)

There is little doubt that the central actions of the alkyl phosphates are due to the inactivation of ChE and not to a direct action of the chemicals on effector cells No other enzymes appear to be inhibited and oxygen consumption of nervous tissue is not affected (91) It is also worthy of comment that the convulsions produced by anti-ChE drugs are the only type that can be blocked specifically by low doses of atropine These findings by no means necessitate acceptance of the theory that central synaptic transmission is accomplished by means of chemical mediators However, it would appear difficult to deny the fact that cells within the central nervous system possess receptors that can be stimulated readily by ACh and that this effect can be blocked by atropine and scopolamine Indeed it would be of great interest to study further the central actions of atropine and scopolamine, particularly in relation to chemical mediation in central synaptic transmission

**AXONAL CONDUCTION** The theory that ACh functions not only in synaptic

transmission but also in impulse propagation along nerve axons and muscle fibers has been proposed by Nachmansohn and associates (43). According to the theory, the release of ACh plays an important rôle in the breakdown of membrane resistance leading to depolarization, and its hydrolysis permits repolarization. Inasmuch as the effects of anti-ChEs have been widely used, both in support of and attack upon the theory, the subject will be reviewed in some detail.

If the release of ACh is an important event in depolarization, and its hydrolysis is essential for repolarization, experimental interference with the sequence of chemical events should interrupt conduction. For example, the local application of ACh to a nerve trunk in high concentration should immediately depolarize the membrane. However, Lorentz de N6 (199) observed that nerve impulse propagation was not affected by soaking a medullated nerve in a 0.9 per cent solution of ACh. Nachmansohn attributes this failure to the inability of a quaternary ammonium cation to penetrate myelin (263). To meet this objection, Eccles (83) has demonstrated that the application of  $10^{-2}$  M solutions of ACh and an adequate concentration of anti-ChE fails to block impulse transmission during repetitive stimulation of nonmedullated fibers. He points out that there is incontrovertible evidence that ACh is liberated from postganglionic cholinergic nerve terminals and hence is able to penetrate an activated nerve membrane. Therefore, Nachmansohn's objections could not apply to nonmedullated fibers.

According to Nachmansohn's theory, the loss of ChE activity in nerve fibers should also interfere with the propagation of the impulse by leading rapidly to persistent depolarization. The demonstration that the highly lipid-soluble DFP inhibited ChE irreversibly offered a unique opportunity to investigate this point. Nachmansohn and associates (45) and Crescitelli and coworkers (64) simultaneously and independently conducted experiments based upon the premise that physostigmine and DFP should block axonal conduction, the former reversibly, the latter irreversibly. Both groups demonstrated that the application of high concentrations of either anti-ChE could block conduction, but the action of DFP could be readily reversed by washing provided the exposure was not of too long duration. To account for this, Nachmansohn and associates demonstrated that whereas the inhibition of ChE activity by DFP was immediate, irreversible inactivation was a delayed event, the duration of which was a function of concentration of inhibitor and temperature (240, 241). In a series of papers, they have related reversible and irreversible nerve block by DFP to the above functions (44, 45, 46, 97, 131). In other words, if nerves are exposed under conditions which would lead to an irreversible inactivation of cholinesterase, impulse propagation cannot be restored. On the other hand, the block produced by physostigmine can be reversed after long exposure of the nerve to high concentrations of this reversible ChE inhibitor.

The parallelism between the irreversible inactivation of ChE and irreversible nerve block loses much of its significance when one examines experiments designed to dissociate the two phenomena, a fact that has been emphasized by Eccles (84). In the experiments of Crescitelli and associates frogs were injected

with enormous amounts of DFP (2.0 grams per kilogram) and their sciatic nerves removed after one or two hours. No disturbance in conduction could be revealed when compared with control nerves, despite the fact that no ChE activity could be detected by the conventional Warburg technic. Similarly, Boyarsky and coworkers (25) exposed frog sciatic nerves to solutions of DFP (0.003 M) in peanut oil for three hours. Conduction was unimpaired, yet no ChE activity could be detected by incubating the ground nerve with ACh and determining the rate of disappearance of the substrate by bioassay. Nachmansohn has objected to each of these experiments on the basis of the technics employed. He claims that the manometric procedure is not sufficiently sensitive to reveal the small residuum of ChE activity that would support nerve conduction. The objection to Boyarsky's technic was based upon the large amount of substrate employed relative to that which would be expected to be hydrolyzed. Nachmansohn and coworkers repeated both of the above types of experiments using the bioassay technic to determine the hydrolysis of small amounts of ACh (97). They employed only 100  $\mu$  gm of substrate per 100 mgm of ground nerve. This mixture was incubated for three hours. Solutions of ACh containing no protein served as controls. At the end of the incubation period, both types of solutions were treated with protein precipitants and the ACh content of the supernatant fluid determined by bioassay. The results indicated that enough ACh had disappeared from the experimental mixture to assign to the nerves seven to eight per cent or more of their original ChE activity. Ignored or uncontrolled by this technic was the possible effect of the protein precipitate in removing the small amount of ACh that disappeared during the procedure as well as the fact that many non-specific enzymes relatively insensitive to inhibition by DFP could readily have destroyed the ACh inasmuch as the ratio of crude enzyme source to substrate was 1000:1 on a weight basis.

Nachmansohn's criterion of the amount of ChE activity necessary to maintain conduction is also subject to interesting variations. For example, he maintains that the frog sciatic nerve can conduct normally when its ChE content is so low as to permit detection only by most elaborate procedures (97), whereas conduction in the lobster cord is markedly reduced or completely blocked when there is still 38 per cent and 21 per cent of esterase activity remaining, respectively (45). If one defines the amount of ChE activity compatible with axonal transmission as that amount which remains, no matter how small, when axons conduct, and defines the amount of ChE activity incompatible with axonal transmission as that amount which remains, no matter how large, when axons fail to conduct, there might possibly be some validity in the Nachmansohn hypothesis. However, the reviewers doubt very much that the frog nerves employed in the above experiments possessed any ChE activity and consider the methods employed by Nachmansohn to salvage some activity in support of his theory inadequately controlled.

The experiments of Nachmansohn and coworkers have been severely criticized on the basis that the high concentration of DFP or physostigmine (circa 0.1 M) required in the fluid bathing the nerve in order to block conduction is

many thousand times that necessary to inhibit ChE. Nachmansohn has attempted to explain this by postulating that the highly lipid-soluble DFP, which should readily penetrate myelin, is present in the nerve axoplasm in only minute amounts. To support this, he has measured the DFP content of the extruded axoplasm of squid giant axons that had been exposed to a solution containing 1 mgm per cc of the inhibitor and found it to be in the order of only  $1 \mu\text{gm per cc}$  (97). The DFP content was measured by determining the anti-ChE activity of the axoplasm. This method is entirely inapplicable inasmuch as Brauer has shown that in the reaction between DFP and ChE, both enzyme and inhibitor are destroyed. Moreover, DFP also reacts with globulins and is thereby inactivated. For example, DFP reacts with various tissue constituents so avidly that when it is injected into the radial artery it is completely inactivated by the tissues of the forearm provided there is a brief period of venous occlusion. Therefore, the technic employed by Nachmansohn would not be applicable unless a long period of equilibration permitted all the cellular constituents capable of reacting with DFP to do so. In contrast to DFP, Nachmansohn found that eserine, which is not inactivated by reacting with ChE, distributed itself equally between the axoplasm and the fluid of the bath at concentrations up to  $10^{-3} \text{ M}$ . This is many times the concentration necessary to inhibit ChE and yet concentrations of this order or higher are necessary to block conduction. Nachmansohn explains this discrepancy by postulating a low dissociation constant of the tertiary amine within the axoplasm. Putting all of Nachmansohn's findings together one is faced with the conclusion that DFP, which is somewhat more active than eserine in blocking nerve conduction, attains a concentration within the axoplasm only 1/1000 that of eserine at the time of block. This finding is scarcely compatible with the fact that eserine is a more active inhibitor of ChE than is DFP.

A seemingly irrefutable objection to the Nachmansohn theory has been raised by Toman and coworkers (297). They stated that the logic of the ACh hypothesis demands that anti-ChEs produce conduction block by the depolarizing action of accumulated ACh. They then performed the simple, decisive experiment of measuring action potentials and demarcation potentials during the course of nerve block with DFP and eserine. Conduction failure occurred without depolarization, a phenomenon that is also characteristic of block by local anesthetics. Furthermore, they argued that according to the ACh hypothesis one would expect signs of excitation in nerves treated with sub-blocking doses of anti-ChEs. However, the least detectable action of DFP or eserine was to increase the threshold. Therefore, in common with the local anesthetics, the anti-ChEs appear to block conduction by interfering with those chemical events that lead to depolarization of the axon and not repolarization as the ACh hypothesis would demand. It is not unlikely that physostigmine acts in a manner exactly analogous to the tertiary amine local anesthetics. One could also postulate that DFP acts by the same mechanism in that presumptive evidence was presented above that eserine and DFP combine with the same moiety of the ChE molecule. The possibility exists, therefore, that the local anesthetics and eserine can react re-



versibly and DFP irreversibly with some substance in the nerve axon, the integrity of which is necessary for depolarization to occur. A common site of reaction of DFP and physostigmine with some substance other than ChE essential for axonal conduction would readily explain the observation of Nachmansohn (234) that the presence of physostigmine prevents the irreversible block of conduction produced by DFP.

The reviewers are of the opinion that the above evidence largely discredits the hypothesis that the release and hydrolysis of ACh are intimately associated with axonal transmission. On the other hand, there is little doubt that ACh can stimulate autonomic effector cells and initiate a propagated impulse in ganglion cells and at the motor endplate of striate muscle. One might generalize, therefore, that ACh is concerned with depolarizing phenomena which are non-propagated and that other mechanisms are involved in conduction.

**TOXICITY** The signs and symptoms of poisoning from the reversible anti-ChEs and the methods of treatment are too well known to warrant review. Attention will therefore be focused upon the toxic effects of the irreversible inhibitors of ChE. Furthermore, the availability of the alkyl phosphates has permitted, for the first time, a study of the chronic effects of reduced tissue cholinesterase activity.

**Acute toxicity** There is little doubt that the alkyl phosphates cause death by inactivating ChE and have no direct action on effector cells. Lethal doses invariably reduce tissue ChE (brain) to levels which are too low for normal physiological function (170, 211, 236). Furthermore the toxicity of the individual alkyl phosphates can be related to their activity in inhibiting ChE *in vitro* (163). The alkyl phosphates can cause death by three distinct mechanisms: (1) excessive stimulation of autonomic effector cells, (2) stimulation followed by paralysis of striate muscle and (3) central stimulation followed by depression. These will be called the muscarinic, nicotinic and central actions, respectively. The various alkyl phosphates differ with respect to the relative intensity of the three types of actions which they elicit. Furthermore species vary in their response. In the present state of our knowledge one can do little more than present in a general manner the toxic reactions to the group as a whole, and the most feasible methods for their management. The treatment of poisoning is of great practical importance because several of the alkyl phosphates are rather widely employed as insecticides.

The muscarinic actions of all the alkyl phosphates are so intense as to lead rapidly to death. Bronchial constriction and bronchorrhea are prominent features of the lethal syndrome in animals (60) but bradycardia, heart block and circulatory collapse undoubtedly contribute greatly. The muscarinic effects can be prevented by atropine. Therefore when this is the prominent feature of poisoning, atropine alone can protect animals from one or more lethal doses.

The alkyl phosphates also have a prominent nicotinic action. Excess stimulation of the motor endplate leads to muscle tremors and fasciculation. The high concentration of ACh rapidly results in muscular paralysis, and death occurs

when the respiratory muscles become involved. Protection against the nicotinic actions of the alkyl phosphates is afforded by agents which block conduction at the neuromuscular junction. Curare has not been adequately studied in this regard. However, the magnesium ion is very effective, and the parenteral administration of magnesium salts protects animals from the nicotinic actions of alkyl phosphates which otherwise would be fatal (214, 227).

Certain of the alkyl phosphates have such a prominent central action that death from minimal lethal doses results from central stimulation followed by depression. The provocative fact that therapeutic doses of atropine can prevent excessive central stimulation has already been discussed.

As an example of species differences, the response of the cat and the rabbit to DFP may be cited. The intravenous  $LD_{50}$  in the two species are 1.6 mgm and 0.4 mgm per kg, respectively (153). In the cat central and muscarinic actions are prominent and atropine alone protects 86 per cent of animals against an  $LD_{100}$  but none against two times the  $LD_{100}$ . The administration of magnesium salts alone provides only slight protection against an  $LD_{100}$ . However, a combination of  $Mg^{++}$  and atropine protects 40 per cent of animals against two times an  $LD_{100}$  (227). In the case of the rabbit, nicotinic and muscarinic actions are equally prominent. Therefore neither atropine nor  $Mg^{++}$  alone is an effective antagonist. However, a combination of both agents can save a high percentage of animals from lethal doses (214). The possibility that  $Mg^{++}$  also contributes to the control of the central actions cannot be ignored.

On the basis of animal studies, certain recommendations can be made for the treatment of human poisoning by the alkyl phosphates. Atropine should be given immediately in maximal dosage (2.0 mgm) by the intravenous route if possible. If muscular fasciculation is prominent, consideration should be given to the use of neuromuscular blocking agents. Theoretically they should stop fasciculation and increase muscle strength. However, although Harvey and associates were able to convert the abnormal electromyogram produced by DFP to a relatively normal one by the use of curare (see above), the muscular weakness was not affected, possibly because the dose of curare was excessive (140). Certainly curare should only be employed by those experienced in its use and with adequate provisions for maintaining respiration by mechanical means if necessary. The effectiveness of  $MgSO_4$  in experimental animals suggests its use in human poisoning. The intramuscular injection of 10 cc of a 25 per cent solution per 100 pounds of body weight is probably a safe procedure, especially if a solution of a calcium salt is available to antagonize any excessive effect on the neuromuscular junction.

*Chronic poisoning* The discovery of the irreversible anti-ChEs provided the opportunity to study the physiological effects of a chronic reduction in the concentration of tissue ChE. Such studies have been conducted in rats, dogs and monkeys, in which DFP was administered over periods ranging up to six months (171). Animals in which the level of ChE activity was only moderately depressed exhibited few signs and symptoms, and no changes occurred in the formed elements of the blood or in the blood chemistry. When dogs were given doses

sufficient to elicit nicotinic and muscarinic responses and such doses were repeated twice weekly for several months, functional disturbances of smooth and striate muscle occurred which persisted after the drug was discontinued. The first effect on striate muscle was the appearance of fasciculations in the tongue. These then spread to other muscles. Muscle weakness of the hind legs eventually leading to paralysis followed within a period of a few weeks. Complete paralysis developed within three months and showed no significant improvement after cessation of drug administration. Outstanding responses of autonomic effector cells were limited to the urinary bladder and the cardiac sphincter. The effect on the bladder was manifested by urinary incontinence. The animals developed constant dribbling and at autopsy the bladders of most were small and contracted. The action on the cardiac sphincter was the ultimate cause of death. So marked and sustained was the response at this site that the animals eventually were unable to retain even liquid food and regurgitated their undigested meals at varying periods after ingestion. Fluoroscopic and postmortem examinations revealed marked dilatation of the esophagus.

The nervous mechanism responsible for the production of cardiospasm has not been clearly defined. At least some of the fibers concerned are apparently cholinergic, since the cardiospasm produced by stimulation of the peripheral vagus in dogs was increased after the administration of physostigmine (189). That the vagus also carries inhibitory fibers is inferred by the fact that chronic cardiospasm can be produced by vagotomy (168). This subject has been discussed in some detail by Lehman (189).

The paralyzing effect of DFP on striate muscle has also been observed in cats that received either single large injections (175) or two to six successive injections (158). Fasciculations followed by ataxia and extreme muscular weakness were observed. A recurrent weakness of the hind limbs was evident for as long as 147 days. Studies of muscle function revealed an increased sensitivity to ACh and inability to maintain a tetanus.

**CLINICAL APPLICATIONS** Anti-ChE drugs are widely employed in therapy. However, discussion will be limited to their use in myasthenia gravis, abdominal distention and glaucoma, fields in which the irreversible inhibitors of ChE have received clinical trial. The results obtained to date indicate that further clinical investigations of this group of compounds will result in valued additions to the armamentarium of useful drugs.

*Myasthenia gravis* In 1901, Oppenheim (244) drew attention to the similarity between myasthenia gravis and curare poisoning, in both of which conditions he considered the primary lesion to be a change in the excitability of the motor end-plates. The confirmation of this general viewpoint by modern investigators has been discussed in the section on neuromuscular transmission. Six years earlier, Jolly (162) had mentioned physostigmine as a likely drug for treating the disease. Apparently this suggestion remained untested until nearly forty years later when Walker (309) administered physostigmine to a myasthenic patient and obtained temporary but marked improvement. The following year, she reported even

better results with neostigmine (310) When given in conjunction with atropine which controls muscarinic effects, neostigmine has proven to be the most valuable compound available for treating this condition (187, 243, 247, 267, 302). Of the numerous other remedies that have been tested in myasthenia (301), only three appear to be of definite benefit ephedrine (88), potassium chloride (188) and guanidine (225) The results of trials of DFP in the treatment of myasthenia have been disappointing (59, 140) Although this compound produces an increase in strength of longer duration than that following neostigmine, the degree of improvement is less, while undesirable side-actions are more marked The latter are referable chiefly to the gastro-intestinal tract and central nervous system, and are probably largely dependent on the high lipid-solubility of the drug TEPP, however, has been found to have certain definite advantages over neostigmine in the clinical trials reported to date (47, 127) When the dosage schedule was properly adjusted, patients experienced approximately the same degree of muscular power as obtained with neostigmine, and it was maintained much more constantly and with less frequently administered doses The side-effects were not nearly so marked as with DFP and were satisfactorily controlled with atropine in most patients The chief disadvantage found with TEPP was the extremely narrow dosage range compatible with maintenance of satisfactory effects It is to be expected that, as further members of this group are tested, still more satisfactory ones will be found

The subjective and objective improvement which neostigmine produces is so specific in myasthenia gravis that its administration to a suspected case serves as a diagnostic test (304) Viets has modified his original test, which is dependent primarily upon clinical observation, to a more exact fluoroscopic one which is applicable when dysphagia is present (303) Another diagnostic test is based on the marked sensitivity exhibited by myasthenic patients towards small doses of curare (15)

In spite of the numerous investigations that have been aimed at elucidating the basic defect in myasthenia, it remains largely a subject for speculation The studies of Lanari (181), Harvey (139) and others have indicated that there is a deficiency in the amount of ACh reaching the receptors of the muscle fiber following a nerve impulse It has been frequently pointed out that this condition could result from (a) a deficiency in the amount of ACh liberated, (b) an excess of ChE at the neuromuscular junction, or (c) an increased threshold for ACh at the motor endplate, possibly due to some circulating curare-like toxin Although no direct evidence exists in favor of the first two possibilities it cannot be said that they have been disproven (164, 291) Walker (311) has published observations favoring the circulating toxin theory She noted that when a patient with bulbar signs exercised his forearms strenuously, no change occurred in the position of the eyelids as long as circulation in the arms was occluded by means of blood pressure cuffs Shortly after pressure was released, the eyelids drooped markedly and the general weakness increased Wilson and Stoner (317) confirmed this finding, and reported in addition that the serum of myasthenic patients contained an alcohol-soluble substance which blocked transmission in the

isolated frog nerve-muscle preparation. The chief criticism against this type of evidence is the fluctuating character of the signs of weakness in myasthenia, which makes the interpretation of observations during brief periods extremely difficult (194).

The relationship between myasthenia gravis and the thymus gland is most provocative. Since Laqueur and Weigert (184) reported in 1901 on the autopsy finding of a thymus tumor in a patient who died of myasthenia, several authors have noted the frequent association of these two conditions (20, 193, 246). In a routine series of 6000 autopsies, thymic abnormalities were rare (152). Thymectomy has been reported to have a favorable effect on the course of the disease (19, 54) and following the operation objective evidence has been obtained of improved neuromuscular transmission (143). However, such results must be judged conservatively because of the not uncommon occurrence of spontaneous remissions. Torda and Wolff (298, 299) reported that thymus extracts, as well as myasthenic serum, inhibited the synthesis of ACh, but this effect has been denied (290, 312). The subject of the thymus in myasthenia has recently been reviewed by Harvey (138).

Most investigators believe that neostigmine exerts its beneficial effects in myasthenia gravis by virtue of its inhibition of the muscle ChE. The injection of large doses of ACh produces a similar but extremely brief recovery of muscular strength (111). Other theories that have been advanced for the mechanism of action include a shift in muscle potassium (66, 296), a change in the accommodation curve of the motor nerves (292) and a direct action at the neuromuscular junction (254). Stare and Ricketts (279) found abnormally low  $O_2$  consumptions in isolated muscle obtained from two myasthenic patients. Oxygen consumption was increased by the addition of physostigmine, neostigmine or certain other substances. The monograph of Goni (125) is recommended as an excellent review of myasthenia gravis.

*Abdominal distention.* Anti-ChE drugs have long been employed in the treatment of abdominal distention. The prominent actions of DFP on the gastrointestinal tract prompted Grob and coworkers (129) to assess the value of the irreversible anti-ChEs in this condition. The pharmacological actions of DFP on the human bowel have been discussed above. Clinically DFP has proved to be highly effective. The best results were obtained when DFP was used in conjunction with neostigmine and posterior pituitary extract. Under these circumstances the irreversible anti-ChE sensitized the bowel to the other stimulants for long periods. TEPP was much less effective in this respect (127).

*Glaucoma.* In Rodin's (255) account of the history of the use of physostigmine in ophthalmology, he mentions that this drug was first employed for the treatment of glaucoma in 1877 by Laqueur (183). Neostigmine has also been used for controlling intraocular tension in glaucoma, but in general has proven less satisfactory (57, 180). Leopold and Comroe (191) reported highly promising results from a trial of DFP in 78 glaucomatous eyes. A high percentage of eyes which failed to respond to physostigmine or pilocarpine was satisfactorily controlled with DFP, and in all cases, as would be expected, DFP required much less

frequent instillation than the other preparations. It is interesting to note, however, that miosis was maintained, on the average, for a shorter period following DFP in glaucomatous than in normal eyes. This raises the possibility that in glaucoma ChE may be regenerated more rapidly, or that the iris sphincter is less sensitive to ACh. The superiority of DFP to physostigmine appeared most striking in chronic simple glaucoma, glaucoma with aphakia, and glaucoma secondary to uveitis, although there were only six cases in the last category. Undesirable side-effects included visual blurring, eye and brow ache, spasm of accommodation and pericorneal injection. Occasionally, a transient rise in tension was recorded. Essentially similar results were obtained by McDonald (212) and von Sallmann and Stone (307). Dunphy (73) reported a somewhat smaller percentage of favorable responses with DFP and, while considering it the drug of choice in glaucoma with aphakia, he advised against its use in congestive glaucoma, in cases following uveitis, or in cases with shallow anterior chambers. Marr (204) considered that only 16 per cent of his cases were controlled successfully by DFP. He administered the drug only in glaucomatous eyes which were refractory to all other miotics, and established extremely stringent criteria for satisfactory control. In one case, retinal detachment occurred, presumably as the result of the marked ciliary spasm produced by DFP. Marr has obtained essentially the same results in glaucoma with TEPP as with DFP (205), but the use of the former drug led to a high incidence of irritation and sensitization of the ocular tissues.

#### EPILOGUE

This review cannot be concluded without a few words of apology from the reviewers. It is obvious that the actions of the anti-ChE drugs touch upon so many problems in pharmacology and physiology that a complete discussion of this group of compounds would be beyond the scope of the present undertaking. One might then pertinently inquire what dictated the choice of material. Unfortunately, no ready answer is available. In general, an attempt has been made to point out the many sites of action of ACh and anti-ChE drugs and the difficulties which arise when one tries to relate specifically a pharmacological response to the inhibition of an enzyme. Certainly a complete understanding of the reactions between enzyme and inhibitor *in vitro* and *in vivo* as well as a knowledge of the reactions of the inhibitor with substances other than the specific enzyme which it inhibits is essential before proper interpretations can be drawn. Many of the controversial issues discussed in this review depend upon such ancillary knowledge for their ultimate solution.

Those who have worked in the field of the toxicology of the anti-ChE drugs have been impressed with their high toxicity. Undoubtedly this is the result both of the great susceptibility of ChE to inhibition and the important physiological functions of the enzyme. It is obvious from the most casual studies of the potent lipid-soluble anti-ChEs that ACh has many sites of action. These include, in addition to autonomic effector cells, the motor endplate of skeletal muscle and the autonomic ganglion cells, as well as numerous other possible sites

in cortical, sub-cortical and spinal areas. The exact relationship between chemical and electrical events at many of these sites awaits elucidation. Certainly the anti-ChE drugs are destined for a major role in such fundamental studies and can provide much useful information if employed with a complete understanding of their chemistry and pharmacology.

The discovery of the anti-ChE activity of the alkyl phosphates represents a major advance in neuropharmacology and neurophysiology. Not only is their property of irreversible inhibition a unique one, but also their chemical constitution and physical properties are so different from those of the reversible inhibitors that many new pharmacological and therapeutic applications are possible. As this new group of compounds expands and is further explored it is hoped that a degree of specificity of action will be attained which will increase the therapeutic value of cholinergic drugs. Promising advances in this direction have already been made.

*Acknowledgments* The authors wish to take this opportunity to express their appreciation to Drs. S. W. Kuffler and J. L. Lilenthal, Jr., for their criticisms of certain portions of this review and for their valued suggestions.

#### REFERENCES

1. ACHESON, G. H., LANGOIR, J. L. AND STANBURY, J. B. Sensitivity of skeletal muscle to intra arterial acetylcholine in normal and myasthenic man. *J Clin Investigation*, 27: 439-445 1948.
2. ADAMS, D. H., AND THOMPSON, R. H. S. The selective inhibition of cholinesterases. *Biochem J*, 42: 170-175, 1948.
3. ADRIAN, E. D., FELDBERG, W., AND HILBY, B. A. Cholinesterase inhibiting action of fluorophosphates. *Brit J Pharmacol*, 2: 50-53, 1947.
4. AZEGLIMANN, J. A. AND REINERT, M. Pharmacological action of some analogues of physostigmine. *J Pharmacol & Exper Therap*, 43: 413-444 1931.
5. ALLES, G. A., AND HAWES, R. C. Cholinesterases in the blood of man. *J Biol Chem*, 133: 375-390, 1940.
6. ALLES, G. A. AND HAWES, R. C. Cholinesterases. *Science* 100: 75 1944.
7. ANDERSON, H. K. The paralysis of involuntary muscle. Part III. On the action of pilocarpine, physostigmine and atropine upon the paralyzed iris. *J Physiol* 33: 414-433 1905.
8. ANDREWS, H. L. The effect of morphine and prostigmine methylsulfate on measurements of pain threshold. *J A. M. A.*, 129: 525-527 1940.
9. AUGUSTINSSON, K. B. Cholinesterases in some marine invertebrates. *Acta physiol Scandinav* 11: 141-151 1946.
10. AUGUSTINSSON, K. B. Cholinesterases. A Study in comparative enzymology. *Acta physiol Scandinav* 15: Supplementum 15: 1-182 1948.
11. BACQ, Z. M. Action de DFP sur le rectus isolé d'amphibien et l'intestin isolé de mammifère. *Compt rend Soc. de biol*, 141: 857-859 1947.
12. BACQ, Z. M. AND BROWN, G. L. Pharmacological experiments on mammalian voluntary muscle in relation to the theory of chemical transmission. *J Physiol*, 89: 45-60 1937.
13. BECK, I. T. AND FROMMELT, E. L'influence de la prostigmine sur la fréquence de l'excitation électrique capable de créer le tétanos musculaire. *Arch Internat de pharmacodyn et de therap* 76: 95-101 1948.
14. BECKETT, S. AND GELLHORN, E. Role of acetylcholine in the activity of sensorimotor and suppressor areas of the cortex. *Am J Physiol*, 153: 113-120 1948.
15. BENNETT, A. E. AND CASH, P. T. Curare sensitivity in myasthenia gravis: a new diagnostic test and approach to causation. *Arch Neurol & Psychiat*, 49: 537-547, 1943.
16. BERNHEIM, F. The interaction of drugs and cell catalysts. Burgess Publishing Co., Minneapolis 1945.
17. BERNHEIM, F. AND BERNHEIM, M. L. C. Action of drugs on the cholinesterase of the brain. *J Pharmacol & Exper Therap* 57: 427-436 1936.
18. BERNHEIM, F. AND BERNHEIM, M. L. C. The hydrolysis of homatropine and atropine by various tissues. *J Pharmacol & Exper Therap*, 64: 209-216 1938.
19. BLALOCK, A., HARVEY, A. M., FORD, F. R. AND LILENTHAL, J. L. JR. The treatment of myasthenia gravis by removal of the thymus gland preliminary report. *J A. M. A.* 117: 1529-1533 1941.
20. BLALOCK, A., MASON, M. F., MORGAN, H. J. AND RIVEN, S. S. Myasthenia gravis and tumors of the thymic region. *Ann. Surg* 110: 544-561, 1939.
21. BLANCHER, H., CHOU, T. C., AND WANDA, I. The inhibition of esterases by paludrine. *Brit. J Pharmacol*, 2: 116-120, 1947.

- 22 BLOCH, H Über die Spezifität des Inhibitors bei der Esterase-Hemmung durch Tri-o-kresyl phosphat *Helv chim Acta*, 26 733-739, 1943
- 23 BOHANSKY, O Cholinesterase *Ann New York Acad Sci.*, 47 521-547, 1946
- 24 BORNSTEIN, M B Presence and action of acetylcholine in experimental brain trauma. *J Neurophysiol.*, 9 349-368, 1946
- 25 BOYARSKY, L L, TORIAS, M, AND GERARD, R W Nerve conduction after inactivation of choline esterase. *Proc Soc Exper Biol & Med*, 64 106-108, 1947
- 26 BRASSFIELD, C R, BIGGINS, C, AND MUSKATMAN, M M The effects of DFP upon the submaxillary gland. *Fed Proc*, 6 80, 1947
- 27 BRAUER, R W Inhibition of cholinesterase activity of human blood plasma and erythrocyte stromata by alkylated phosphorus compounds *J Pharmacol & Exper Therap*, 92: 162-172, 1948
- 28 BRINDLEY, C O Demerol and cholinesterase *Fed. Proc.*, 3: 5, 1944
- 29 BRISCOE, G The antagonism between curarine and prostigmine and its relation to the myasthenia problem *Lancet*, 1 469-472, 1938
- 30 BRONK, D W Synaptic mechanisms in sympathetic ganglia *J Neurophysiol.*, 2 389-401, 1939
- 31 BRONK, D W, TOWSE, S S, BOLANDT, D Y, AND LARRABEE, M G The transmission of trains of impulses through a sympathetic ganglion and in its postganglionic nerves *Am J Physiol.*, 122 1-15, 1938.
- 32 BROWN, G L Action potentials of normal mammalian muscle Effects of acetylcholine and eserine *J Physiol*, 89 220-237, 1937
- 33 BROWN, G L The actions of acetylcholine on denervated mammalian and frog's muscle *J Physiol*, 87 481-481, 1937
- 34 BROWN, G L, BURNS, D, AND FELDBERG, W The effect of DFP on neuromuscular transmission in cat. *J Physiol*, 107 346-354, 1948
- 35 BROWN, G L, DALE, H H, AND FELDBERG, W Reactions of the normal mammalian muscle to acetylcholine and to eserine *J Physiol*, 87 394-424, 1938
- 36 BROWN, G L, AND FELDBERG, W The action of potassium on the superior cervical ganglion of the cat. *J Physiol*, 86 290-305 1936
- 37 BROWN, G L AND HARVEY, A M Reactions of avian muscle to acetylcholine and eserine. *J Physiol.*, 94: 101-117, 1938
- 38 BROWN, G L, AND HARVEY, A M Neuromuscular transmission in the extrinsic muscles of the eye. *J Physiol.*, 99 379-399, 1941
- 39 BUCHTHAL, F, AND LINDHARD, J Transmission of impulses from nerve to muscle fiber *Acta physiol. Scandinau.*, 4 136-148, 1942
- 40 BULBRINO, E Observations on the isolated phrenic nerve diaphragm preparation of the rat *Brit. J Pharmacol.*, 1: 38-61, 1946
- 41 BULBRING, E, AND CHOU, T C The relative activity of prostigmine homologues and other substances as antagonists to tubocurarine *Brit J Pharmacol*, 2 8-22, 1947
- 42 BULLOCK, T H Properties of a single synapse in the stellate ganglion of squid *J Neurophysiol.*, 11: 343-355, 1948
- 43 BULLOCK T H, GRUNDFEST, H, NACHMANSON, D, AND ROTHENBERG, M A Generality of the role of acetylcholine in nerve and muscle conduction. *J Neurophysiol*, 10 11-22, 1947
- 44 BULLOCK, T H, GRUNDFEST, H, NACHMANSON, D, AND ROTHENBERG, M A Effect of di isopropyl fluorophosphate (DFP) on action potential and cholinesterase of nerve *J Neurophysiol.*, 10: 63-78, 1947
- 45 BULLOCK T H, GRUNDFEST, H, NACHMANSON, D, ROTHENBERG, M A., AND STERLING, K. Effect of di-isopropyl fluorophosphate (DFP) on action potential and cholinesterase of nerve *J Neurophysiol*, 9: 253-260 1946
- 46 BULLOCK, T H NACHMANSON, D, AND ROTHENBERG, M A. Effects of inhibitors of choline esterase on the nerve action potential *J Neurophysiol.*, 9 9-23 1946
- 47 BURGEN, A S V, KEELER, C A, AND McALPINE, D Tetra-ethyl pyrophosphate in myasthenia gravis *Lancet*, 1: 519-521, 1948
- 48 CALMA, I, AND WRIGHT, S Action of intrathecally injected eserine on the spinal cord of the cat *J Physiol*, 106: 80-94, 1947
- 49 CANTONI, G L, AND LOEWI, O Inhibition of cholinesterase activity of nervous tissues by eserine in vivo *J Pharmacol & Exper Therap*, 81 67-71, 1944
- 49-A CANNON W B, AND ROSENBLUTH A. Autonomic neuro-effector systems. The Macmillan Co., New York, 1937
- 50 CANNON, W-B, AND ROSENBLUTH A The transmission of impulses through a sympathetic ganglion *Am J Physiol*, 119: 221-235, 1937
- 51 CAREY, E J Studies on ameboid motility and secretion of motor end plates III Experimental histopathology of motor end plates produced by quinine curare prostigmine acetylcholine, strychnine tetraethyl lead and heat *Am J Path.*, 20 341-393, 1944
- 52 CHASE H F, SCHMIDT, J L, AND BHOTTOCHARYA B K Antagonism of curare action by DFP *Fed Proc.*, 6: 315, 1947
- 53 CHOU, T C, AND DELIO, F J The anti-curare activity of eserine on the superior cervical ganglion of the cat. *Brit J Pharmacol*, 3 113-116 1948
- 54 CLAOWETT, O T, AND EATON, L M Surgical treatment of myasthenia gravis *J Thoracic Surg*, 16 62-80, 1947



55. CLARK, A. J., AND RAVENTOS, J. Antagonism of acetylcholine and of quaternary ammonium salts. *Quart J Exper Physiol*, 26 375-392 1937
56. CLARK, A. J., AND RAVENTOS, J. Hydrolysis of acetylcholine by tissues. *Quart. J Exper Physiol*, 28 155-176, 1938
57. CLARK, S. T. Mecholyl and prostigmine in the treatment of glaucoma. *Am J Ophth*, 22 249-257 1935
58. COHEN, S. L. The effect of DFP upon the response of the turtle heart to vagus stimulation. *Fed Proc.*, 6 52, 1947
59. CONROE, J. H., TODD, J., GAMMON, G. D., LEOPOLD, I. H., KOELLE, G. B., BODANSKY, O., AND GILMAN, A. The effect of DFP upon patients with myasthenia gravis. *Amer J M Sc.*, 312 641-651 1946
60. COPPÉE, G. La transmission neuro-musculaire curarisation, d'curarisation et renforcement à la jonction myoneurale. *Arch internat de Physiol*, 53 327-507 1943
61. COPPÉE, G., AND BACQ, Z. M. Action de DFP sur la préparation neuromusculaire de grenouille. *Compt rend. Soc. de biol.*, 141 559-531 1947
62. COWAN, S. L. The initiation of all-or none responses in muscle by acetylcholine. *J Physiol*, 83 3P-5P, 1936
63. COWAN, S. L. The action of eserine-like and curare-like substances on the responses of frog a nerve-muscle preparations to repetitive stimulation. *J Physiol*, 93. 215-262 1938.
64. CRESCITELLI, F., KOELLE, G. B., AND GILMAN, A. Transmission of impulses in peripheral nerves treated with diisopropyl fluorophosphate (DFP). *J Neurophysiol*, 9 241-252 1946
65. CUMMINGS, J. N. The potassium content of muscle in disease. *Brain*, 62 153-156 1939
66. CUMMINGS, J. N. The role of potassium in myasthenia gravis. *J Neurol & Psychiat.*, 3 115-122, 1940
67. DALE, H. H., FELDBERG, W., AND VOGT, M. Release of acetylcholine at voluntary motor nerve endings. *J Physiol.*, 86 353-350, 1936
68. DARBOW, C. W., GREEN, J. R., DAVIS, E. W., AND GAROL, H. W. Parasympathetic regulation of high potential in the electroencephalograph. *J Neurophysiol*, 7 217-226 1944
69. DAYAL, C., MANRY, C. H., AND SEEVERS, M. H. On the pharmacology of hexaethyl tetraphosphate. *J Pharmacol. & Exper Therap*, 92. 173-168, 1948.
70. DENNY BROWN, D. B., AND PENNPACKER, J. B. Fibrillation and fasciculation in voluntary muscle. *Brain*, 61. 311-334, 1938.
71. DUBOIS, K. P., ERWAY, W. F., AND BYERUM, R. U. A comparison of cholinesterase inhibitors in vitro and in vivo. *Fed. Proc.*, 6 326, 1947
72. DUBOIS, K. P., AND MANOUV, G. H. Effect of hexaethyl tetraphosphate on choline esterase in vitro and in vivo. *Proc. Soc. Exper Biol. & Med.*, 64: 137-139, 1947
73. DUNPHY, E. B. DFP in glaucoma. *Bull. New England Med. Center*, 9- 103-106, 1947
74. EADIE, G. S. The inhibition of cholinesterase by physostigmine and prostigmine. *J Biol. Chem*, 146 55-63, 1942
75. EASBON, L. H., AND STEDMAN, E. The absolute activity of choline-esterase. *Proc. Roy Soc.*, B121 142-164 1936.
76. EASBON, L. H., AND STEDMAN, E. The specificity of cholinesterase. *Biochem. J.*, 31 1723-1729 1937
77. ECCLES, J. C. Synaptic transmission through a sympathetic ganglion. *J Physiol*, 81 8P, 1934
78. ECCLES, J. C. The discharge of impulses from ganglion cells. *J Physiol*, 91 1-22, 1937
79. ECCLES, J. C. The nature of synaptic transmission in a sympathetic ganglion. *J Physiol.* 103 27-54 1944.
80. ECCLES, J. C. Electrical hypothesis of synaptic and neuromuscular transmission. *Nature*, 156 650-653, 1945
81. ECCLES, J. C. Synaptic potentials of motoneurons. *J Neurophysiol*, 9- 87-120, 1946
82. ECCLES, J. C. An electrical hypothesis of synaptic and neuromuscular transmission. *Ann. New York Acad. Sci.*, 47 429-455 1946
83. ECCLES, J. C. Acetylcholine and synaptic transmission in the spinal cord. *J Neurophysiol.*, 10 197-204, 1947
84. ECCLES, J. C. Conduction and synaptic transmission in the nervous system. *Ann. Rev Physiol*, 10 93-116 1948.
85. ECCLES, J. C., KATZ, B. AND KUFFLER, S. W. Nature of the "endplate potential" in curarized muscle. *J Neurophysiol.*, 4. 362-387, 1941
86. ECCLES, J. C., KATZ, B., AND KUFFLER, S. W. Effect of eserine on neuromuscular transmission. *J Neurophysiol.*, 5 211-230 1942
87. ECCLES, J. C., AND MACFARLANE, V. W. Unpublished observations, 1947. Quoted in reference 84.
88. EDGEWORTH, H. A report of progress on the use of ephedrine in a case of myasthenia gravis. *J. A. M. A.*, 94: 1136, 1930
89. ELLIS, S. Enzymic hydrolysis of morphine esters. *Fed. Proc.*, 7 216 1948.
90. ELLIS, S. Studies on physostigmine and related substances. IV. Chemical studies on physostigmine breakdown products and related epinephrine derivatives. *J Pharmacol & Exper Therap*, 79- 364-372 1943
91. ELLIS, S. Effect of diisopropyl fluorophosphate (DFP) on the  $QO_2$  of the rabbit cerebral cortex. *Proc. Soc. Exper Biol. & Med.*, 69 353-364, 1948.
92. ELLIS, S., KRAVER, O., AND PLACHTE, F. L. Studies on physostigmine and related substances. III. Breakdown products of physostigmine their inhibitory effect on cholinesterase and their pharmacological action. *J Pharmacol. & Exper Therap*, 79 309-319 1943
93. ELLIS, S., PLACHTE, F. L. AND STRAUS, O. H. Studies on physostigmine and related substances. II. The destruction of physostigmine in buffered solutions and in serum. *J Pharmacol & Exper Therap*, 79- 293-303 1943
94. EMMELIN, N. G. AND JACOBSON, D. Some effects of acetylcholine, eserine and prostigmine when injected into the hypothalamus. *Acta physiol. Scandinav*, 9- 97-111 1945.

- 95 EMMELIN, N G, AND MACINTOSH, F C Some conditions affecting the release of acetylcholine in sympathetic ganglia and skeletal muscles *Acta physiol Scandinau*, 16: Suppl 53, 17-18, 1948.
- 96 ENGLEHART, E, AND LOEWI, O Fermentative acetylcholinaspaltung im blut und ihre hemmung durch physostigmine *Arch exper Path u Pharmacol*, 159 1-13, 1930.
- 97 FELD, E A, GRUNDFEST, H, NACHMANSOHN, D, AND ROTHENBERG, M A Effect of di isopropyl fluorophosphate (DFP) on action potential and cholinesterase of nerve IV *J Neurophysiol*, 11: 125-137, 1948.
- 98 FELDBERG, W Present views on the mode of action of acetylcholine in the central nervous system *Physiol. Rev.*, 25 596-642, 1945
- 99 FELDBERG, W, AND GADDUM, J H The chemical transmitter at synapses in a sympathetic ganglion. *J Physiol*, 81: 305-319, 1934
- 100 FELDBERG, W, MINZ, B, AND TADZUMURA, H The mechanism of the nervous discharge of adrenaline. *J Physiol*, 81 286-304, 1934
- 101 FELDBERG, W, AND VARTAINEN, A. Further observations on the physiology and pharmacology of a sympathetic ganglion *J Physiol*, 83 103-128, 1934.
- 102 FENG, T P Studies on the neuromuscular junction. XVIII The local potentials at neuromuscular junctions induced by single and multiple volleys *Chin J Physiol*, 15 337-404, 1940
- 103 FENG, T P, LI, T H, AND TING, Y C Studies on the neuromuscular junction. XV The inhibition following eserine-potentialized and post-tetanicly facilitated responses of mammalian muscles *Chin J Physiol*, 16: 329-356, 1939
- 104 FILLENZ, M, AND HANAFIN, M : Acetylcholine and neuromuscular transmission *J Neurophysiol*, 10. 169-195, 1947
- 105 FINERTY, J C Potentiation of response of rectus abdominis of frog to acetylcholine and DFP *Fed. Proc.*, 6 103, 1947
- 106 FINERTY, J C, AND GESSELL, R The effect of CH on humoral stimulation of striated muscle and its application to the control of breathing *Am. J Physiol.*, 145 1-15, 1943.
- 107 FISHER, S M, AND STAVRAKY, G W The effects of acetyl beta methylcholine in human subjects with localized lesions of the central nervous system *Am J M. Sc.*, 208: 371-380, 1944.
- 108 FLENNMARK, S, AND WRAMNER, T The analgetic action of morphine, eserine and prostigmine studied by a modified Hardy Wolff Goodell method *Acta physiol Scandinau*, 9 89-96, 1945
- 109 FORSTER, F M, BORKOWSKI, W J, AND MCCARTER, R. H Depression of the cerebral cortex induced by applications of acetylcholine *Fed Proc*, 5 28, 1946
- 110 FORSTER, F M, AND MCCARTER, R H Spread of acetylcholine induced electrical discharges of the cerebral cortex. *Am J Physiol*, 144: 169-173, 1945
- 111 FRASER, F R, McGEORGE, M, AND MURPHY, G E Action of choline esters in myasthenia gravis *Clin. Sc.* 3: 77-89, 1937
- 112 FREY, J S, AND GESSELL, R. A comparative study on the effects of several anti-cholinesterases on central nervous integration *Fed Proc.*, 7 37, 1948
- 113 GADDUM, J H, AND WILSON, A Treatment of myasthenia gravis with DFP *Nature*, 159: 680-681 1947
- 114 GALEHR, O, AND PLATTNER, F Über das schicksal der acetylcholine im blute. *Arch f d. ges. Physiol*, 218: 488-505, 1927
- 115 GESSELL, R, BRADFELD, C R, AND HAMILTON, M A An acid neurohumoral mechanism of nerve cell activation *J Physiol*, 136 604-608, 1942
- 116 GESSELL, R, AND HANSEN, E T Anti cholinesterase activity of acid as a biological instrument of nervous integration. *Am J Physiol*, 144: 126-163, 1945
- 117 GILMAN A The effect of drugs on nerve activity *Ann New York Acad Sc.*, 47 549-558, 1946.
- 118 GLICK, D Studies on the specificity of cholinesterase *J Biol Chem*, 125 729-739, 1938.
- 119 GLICK, D Further studies on the specificity of cholinesterase *J Biol Chem*, 130: 527-534, 1939
- 120 GLICK, D Some additional observations on the specificity of cholinesterase *J Biol. Chem*, 137 357-362, 1941
- 121 GLICK, D The controversy on cholinesterases *Science*, 102 100-101, 1945
- 122 GOLDFINGER, D, AND WOIKA, P H The electrocardiographic effects of prostigmin. *Am J M. Sc.*, 212: 418-422, 1946
- 123 GOLDSTEIN, A The mechanism of enzyme-inhibitor-substrate reactions, illustrated by the cholinesterase-physostigmine-acetylcholine system *J Gen Physiol*, 27 529-580, 1944
- 124 GOLDSTEIN, A Inhibitors of purified human plasma cholinesterase *Fed. Proc.*, 7: 223, 1948.
- 125 GONI, A R Myasthenia Gravis *The Williams & Wilkins Co*, Baltimore 1946
- 126 GREEN, R E, MCKAY, E A, AND KNOP, S The action of DFP on the caliber of the bronchial tree in isolated lungs *Fed Proc.*, 6 334 1947
- 127 GROS, D, AND HARVEY, A M *Bull Johns Hopkins Hosp* (In press)
- 128 GROS, D, HARVEY, A M, LANGWORTHY, O R, AND LILIENTHAL, J L, JR. The administration of di isopropyl fluorophosphate to man III Effect on the central nervous system with special reference to the electrical activity of brain *Bull Johns Hopkins Hosp*, 81: 257-268, 1947
- 129 GROS, D, LILIENTHAL, J L, JR, AND HARVEY A M The administration of DFP to man. II Effect on intestinal motility and use in the treatment of abdominal distention. *Bull. Johns Hopkins Hosp*, 81: 245-256, 1947
- 130 GROS, D, LILIENTHAL J L, JR., HARVEY, A M, AND JONES, B F The administration of di isopropyl fluorophosphate (DFP) to man I. Effect on plasma and erythrocyte cholinesterase, general systemic effects used in study of hepatic function and erythropoiesis, and some properties of plasma cholinesterase *Bull Johns Hopkins Hosp*, 81: 217-244, 1947

- 131 GRUNDFEST H, NACHMANSOHN, D, AND ROTHENBERG M A. Effect of di isopropyl fluorophosphate (DFP) on action potential and cholinesterase of nerve. III J Neurophysiol, 10 155-161 1947
- 132 GUTTON, A., AND MACDONALD M A. Physiology of botulinus toxin. Arch Neurol & Psychiat, 57 578-592 1947
- 133 HAJEK, N M, AND HINES H M. Experimental treatment of muscular spasticity. Fed. Proc., 6 119, 1947
- 134 HALDANE, J B S. Enzymes. Longmans Green & Co, New York 1930
- 135 HARRIS, M M, AND HARRIS R M. Effect in vitro of curare alkaloids and crude curare preparations on "True" and pseudocholinesterase activity. Proc Soc. Exper Biol & Med. 56 223-225 1944.
- 136 HARVEY, A M. The actions of quinine on skeletal muscle. J Physiol, 95 45-67 1939
- 137 HARVEY, A M. The actions of procaine on neuro-muscular transmission. Bull Johns Hopkins Hosp 65 223-238, 1939
- 138 HARVEY, A M. The thymus and myasthenia gravis. Am J Med. 2 549-550 1947
- 139 HARVEY, A M, AND LILIENTHAL, J L, JR. Observations on the nature of myasthenia gravis. The intra-arterial injection of acetylcholine, prostigmine and adrenalin. Bull Johns Hopkins Hosp 69 566-577, 1941
- 140 HARVEY A M, LILIENTHAL, J L, JR. GROSS D JONES B F AND TALBOT S A. The administration of DFP to man. IV The effects on neuromuscular function in normal subjects and in myasthenia gravis. Bull. Johns Hopkins Hosp 81 217-232 1947
- 141 HARVEY, A M LILIENTHAL, J L. JR. AND TALBOT S A. On the effects of the intra arterial injection of acetylcholine and prostigmine in normal man. Bull Johns Hopkins Hosp 69 529-546 1941
- 142 HARVEY A M, LILIENTHAL, J L, JR. AND TALBOT S A. Observations on the nature of myasthenia gravis. The phenomenon of facilitation and depression of neuromuscular transmission. Bull. Johns Hopkins Hosp , 69 547-565, 1941
- 143 HARVEY A M, LILIENTHAL, J L, JR., AND TALBOT S A. Observations on the nature of myasthenia gravis. The effect of thymectomy on neuromuscular transmission. J Clin Investigation 21 579-583 1942
- 144 HARVEY, A M, AND MACINTOSH, F C. Calcium and synaptin transmission in a sympathetic ganglion. J Physiol. 97: 408-416 1940
- 145 HAWKINS, R. D, AND GUNTER, J M. Studies on cholinesterase. 5 The selective inhibition of pseudo-cholinesterase in vivo. Biochem J, 40 192-197 1946
- 146 HAWKINS R. D AND MENDEL, B. True cholinesterases with pronounced resistance to eserine. J Cell. Comp Physiol, 27 62-85, 1946
- 147 HAWKINS, R. D, AND MENDEL, B. Selective inhibition of pseudo-cholinesterase by di isopropylfluorophosphate. Brit J Pharmacol, 2, 173-180 1947
- 148 HENDERSON W R, AND WILSON, W C. Intraventricular injection of acetylcholine and eserine in man. Quart J Exp Physiol. 26 83-95 1936.
- 149 HEYMANS, C, PANNIER, R. AND VERBEKE, R. Influence des anticholinestérases prostigmine, éserine et di isopropyl fluorophosphate, et de l'atropine sur la transmission centrale et périphérique des excitations nerveuses. Arch. internat. de pharmacodyn. et de thérap 72 405-423 1938
- 150 HIMWICH H. E, AND FREEDMAN, A M. DFP Site of injection and variation in response. Fed. Proc., 7 55, 1948
- 151 HONES, R., AND STEINMAN S. E. The effects of acetylcholine and eserine on frog muscle. Am J Physiol 127 470-479, 1939
- 152 HOMBURGER, F.. Changes in thymus in myasthenia gravis observations in series of 6000 autopsies. Arch. Path., 36 371-380 1943
- 153 HORTON R. G KOELLE, G B, McNAMARA, B P, AND PRATT, H. J. The acute toxicity of di isopropyl fluorophosphate. J Pharmacol. & Exper Therap 87 414-420, 1946.
- 154 HOTTINGER, A., AND BLOCH H. Über die Spezifität der cholin-esterase-Hemmung durch Tri-o-kresyl phosphat. Helv chim. Acta 26 142-155, 1943
- 155 HUIDOBRO F. Effect of picrotoxin, strychnine and metrazol (pentamethylenetetrazole) on the action of prostigmine and curare on the neuromuscular junction. J Pharmacol & Exper Therap 86 60-65 1946
- 156 HUIDOBRO F AND JORDAN J J. The action of picrotoxin and of nilethamide (diethylamide of pyridine-3-carboxylic acid) on neuromuscular conductivity. J Pharmacol & Exper Therap 86 49-59 1946
- 157 HUNT, C C. The effect of DFP on neuromuscular transmission. J Pharmacol. & Exper Therap , 91: 77-83 1947
- 158 HUNT C C, AND RIKER, W F JR. The effect of chronic poisoning with DFP on neuromuscular function in the cat. J Pharmacol. & Exper Therap 91 293-305 1947
- 159 HYNK, J BECKETT S., AND GILLBORN, E. Acetylcholine, cholinesterase, eserine and convulsions. Fed. Proc., 6 133 1947
- 160 INOLEFINGER, F J. The modification of intestinal motility by drugs. New England J Med 229: 114-122, 1943
- 161 JACO N T AND WOOD D R. The interaction between procaine, cocaine, adrenalin, and prostigmine on skeletal muscle. J Pharmacol & Exper Therap 82. 63-73 1944.
- 162 JOLLY F. Ueber myasthenia gravis pseudoparalytica. Berl klin Wochr, 37: 1-7, 1895
- 163 JONES H W JR., MEYER, B J AND KAREL, L. The relationship of cholinesterase inhibiting activity to the toxicity of some organo phosphorus compounds. J Pharmacol. & Exper Therap 94 215-220 1948
- 164 JONES, M S AND STADIZ, W C. The cholinesterase content of the muscle of myasthenia gravis and of the serum of four other groups of clinical conditions. Quart. J Exper Physiol, 29 63-67 1939
- 165 KAHLOW, G, AND MACINTOSH, F C. Acetylcholine synthesis in a sympathetic ganglion. J Physiol 96 277-292, 1939

- 166 KENBLER, C J Anti-curare action of certain azo dyes in the frog Fed. Proc., 6 344, 1947
- 167 KINJAKOW, A W Über humorale Übertragung der Erregung von einem Neuron auf das andere Arch. f. d. ges. Physiol., 232 432-443, 1933
- 168 KNIGHT, G C Relation of extrinsic nerves to functional activity of the oesophagus Brit. J. Surg., 22: 185-188, 1934
- 169 KOELLE, G B Protection of cholinesterase against irreversible inactivation by DFP in vitro J. Pharmacol. & Exper. Therap. 88: 232-237, 1946
- 170 KOELLE, G B, AND GILMAN, A The relationship between cholinesterase inhibition and the pharmacological action of DFP J. Pharmacol. & Exper. Therap., 87: 421-434, 1946
- 171 KOELLE, G B, AND GILMAN, A The chronic toxicity of DFP in dogs, monkeys and rats J. Pharmacol. & Exper. Therap., 87 435-443, 1946
- 172 KOELLE, G B, AND KOELLE, E S Serum potassium in dogs unchanged after DFP Unpublished observations
- 173 KOPFANT, T, DILLE, J M, AND LINROAR, C R Studies on synergism and antagonism of drugs, the action of physostigmine on autonomic ganglia J. Pharmacol. & Exper. Therap., 58 103-110, 1936.
- 174 KOPFANT, T, KARZMAR, A. G., AND KING, T O Effect of tetraethylpyrophosphate on sympathetic ganglionic activity Science, 1947, 106: 492-493
- 175 KOSTER, R Synergisms and antagonisms between physostigmine and di isopropyl fluorophosphate in cats. J. Pharmacol. & Exper. Therap., 83: 39-46, 1946
- 176 KRAVER, O, GOLDSTEIN, A, AND PLACHTE, F L Studies on physostigmine and related substances I. Quantitative relationship between dosage of physostigmine and inhibition of cholinesterase activity in blood serum of dogs J. Pharmacol. & Exper. Therap., 80 8-30, 1944
- 177 KROP, S The effect of DFP, di isopropylchlorophosphate, di isopropylphosphite and di isopropylphosphate on the mechanical response of striated frog muscle Fed. Proc., 6: 347, 1947
- 178 KUFFLER, S W Electric potential changes at an isolated nerve-muscle junction J. Neurophysiol. 5: 18-26, 1942
- 179 KUFFLER, S W Specific excitability of the endplate region in normal and denervated muscle J. Neurophysiol., 6: 99-110, 1943
- 179-A KUFFLER, S W Physiology of neuro-muscular junctions electrical aspects Fed. Proc., 7 437-446, 1948.
- 180 KULL, M P Prostigmin in the treatment of glaucoma Am. J. Ophth., 26 57-62, 1943
- 181 LANARI, A Miasthenia gravis y transmisión química neuromuscular Rev. Soc. argent. de biol., 13: 239, 1937
- 182 LANOLEY, J N AND KATO, T The physiological action of physostigmine and its action on denervated skeletal muscle J. Physiol., 49 410-431, 1915
- 183 LAQUEUR, L Ueber Atropin und Physostigmin und ihre Wirkung auf den intraocularen Druck (Ein Beitrag zur Therapie des Glaucoms) Arch. f. Ophth., 23 (part 3) 149-176, 1877
- 184 LAQUEUR, L AND WEIGERT, C Beiträge zur Lehre von der Erb'schen Krankheit ueber die Erb'sche Krankheit. Neurol. Zbl., 20 594-601, 1901
- 185 LARRABEE, M G AND BRONX, D W Prolonged facilitation of synaptic excitation in sympathetic ganglia. J. Neurophysiol., 10 139-154, 1947
- 186 DELAUNENFELS, M W Cholinesterase Science, 98 459, 1943
- 187 LAURENT, L P E AND WALKER, M B Oral and parenteral administration of prostigmine and its analogues in myasthenia gravis Lancet, 1: 1457-1459, 1936
- 188 LAURENT, L P E, AND WALTHER, W W Influence of large doses of potassium chloride on myasthenia gravis. Lancet, 1 1434-1435, 1935
- 189 LEHMANN, G Gastric cardiospasm in the dog Am. J. Physiol., 143 163-168, 1945
- 190 LEOPOLD, I H, AND COMBOS, J H, JR. Effect of neostigmine (prostigmin) and physostigmin upon the denervated iris of the cat Proc. Soc. Exper. Biol. & Med., 69: 382-384, 1945
- 191 LEOPOLD I H, AND COMBOS, J H JR. Use of di isopropylfluorophosphate ("DFP") in treatment of glaucoma Arch. Ophthal., 36 1-18, 1946
- 192 LEOPOLD, I H, AND COMBOS, J H, JR. Effect of DFP on the normal eye Arch. Ophthal., 36: 17-32, 1946.
- 193 LIEVRE, J A Peut-on tenter un traitement chirurgical de la myasthénie Presse méd., 44. 991-992, 1936
- 194 LILIENTHAL, J L, JR., AND HARVEY, A M Personal Communication
- 195 LINSEWEAVER, H, AND BURE, D The determination of enzyme dissociation constants J. Am. Chem. Soc. 56 659-666, 1934
- 196 LOEWI, O, AND NAVRATIL, E Über humorale Übertragbarkeit der Herznervenerwirkung, über das Schicksal des Vagusstoffes Arch. f. d. ges. Physiol., 214. 678-683, 1926
- 197 LORENTE DE NÓ, R Liberation of acetylcholine by the superior cervical sympathetic ganglion and the nodosum ganglion of the vagus Am. J. Physiol., 121 331-349, 1938
- 198 LORENTE DE NÓ, R Release of acetylcholine by sympathetic ganglia and synaptic transmission. Science, 91 501-503, 1940
- 199 LORENTE DE NÓ, R. Effects of choline and acetylcholine chlorides on peripheral nerve fibers. J. Cell. & Comp. Physiol., 24 83-97, 1944.
- 200 MACINTOSH, F C Liberation of acetylcholine by the perfused superior cervical ganglion. J. Physiol., 94: 155-169, 1938
- 201 MACINTOSH, F C Distribution of acetylcholine in peripheral and central nervous systems J. Physiol. 99 436-442, 1941

- 202 MACKWORTH J F AND WEBB F C The inhibition of serum cholinesterase by alkyl fluorophosphonates Biochem J 42 91-95, 1948
- 203 MANNING, C W, LANG J, AND HALL, G E The in vivo effects of eserine on the cholinesterase system J Pharmacol. & Exper Therap 61 350-363, 1937
- 204 MARR, W G The clinical use of DFP in chronic glaucoma Am J Ophthal 30 1423-1426, 1947
- 205 MARR W G Personal Communication
- 206 MARRAZZI, A S, AND JARVIK, N E The differential effects on synaptic transmission and nerve conduction of DFP and atropine Fed Proc., 6 354, 1947
- 207 MASLAND, R L, AND WIGTOV, R S Nerve activity accompanying fasciculation produced by prostigmine. J Neurophysiol., 3 269-275, 1940
- 208 MASSART, L AND DUTAIT, R P Hemmung der acetylcholinesterase durch farbstoffe und durch eserine Enzymologia, 9 364-365, 1941
- 209 MATTHEZ, K. The action of blood on acetylcholine J Physiol, 70 338-348, 1930
- 210 MAZUR, A. Enzyme in animal tissues capable of hydrolyzing phosphorus-fluorine bond of alkyl fluorophosphates J Biol Chem, 164. 271-289 1946
- 211 MAZUR, A., AND BONANSAKY, O Mechanism of in vitro and in vivo inhibition of cholinesterase activity by DFP J Biol Chem., 163 281-276, 1946
- 212 McDONALD, P R Treatment of glaucoma with DFP Am J Ophth, 29 1071-1081, 1946
- 213 McINTYRE, A R., AND KING, R. E d Tubocurarine chloride and cholinesterase Science, 97 69, 1943
- 214 McNAMARA B P, KOELLE, G B, AND GILMAN, A The treatment of di isopropyl fluorophosphate (DFP) poisoning in rabbits J Pharmacol. & Exper Therap, 58 27-33, 1946
- 215 MENDEL, B, AND HAWKINS, R D Removal of acetylcholine by cholinesterase injections and the effect thereof on nerve impulse transmission J Neurophysiol, 6 431-438, 1943
- 216 MENDEL, B, MENDEL, D B, AND RUDNEY H Studies on cholinesterase 3 Specific tests for true cholinesterase and pseudo-cholinesterase. Biochem. J, 37 473-476, 1943
- 217 MENDEL, B, AND RUDNEY, H Studies on cholinesterase 1 Cholinesterase and pseudo-cholinesterase Biochem. J, 37 59-63, 1943
- 218 MENDEL, B, AND RUDNEY, H On the type of cholinesterase present in brain tissue Science 98 201-202, 1943
- 219 MENDEL, B, AND RUDNEY, H The cholinesterases in the light of recent findings Science 100 492-500 1944.
- 220 MENDEL, R., AND RAVIN, A. On the action of prostigmine on the circulatory system. J Pharmacol. & Exper Therap, 72 50-59, 1941
- 221 MERRITT, H. H., AND BRENNER, C The effect of ACh on the electrical activity of the cerebral cortex Trans Am. Neurol. Assoc., 67 152-154, 1941.
- 222 MICHAELIS, L., AND MENTEN, M L Die Kinetik der Invertinwirkung Biochem. J, 49 333-369, 1913
- 223 MILHORAT, A. T, AND ALMY, T P Effect of prostigmine and physostigmine on muscular fibrillations Am J Physiol., 133: P389, 1941
- 224 MILLER, F R Direct stimulation of the hypoglossal nucleus by acetylcholine in extreme dilutions Proc. Soc Exper Biol. Med., 54 255-257, 1943
- 225 MINOT A. S, DONN, K., AND RIVEN S. S. Use of guanidine hydrochloride in the treatment of myasthenia gravis J A. M. A. 113. 553-559, 1939
- 226 MIQUEL, O The action of physostigmine, DFP, and other para-sympathomimetic drugs on the rectus muscle of the frog. J Pharmacol. & Exper Therap 58. 67-71 1945.
- 227 MOORE, W, AND KROP, S Antidotes to poisoning by diisopropylfluorophosphate in cats. J Pharmacol. & Exper Therap, 83 34-38, 1946
- 228 MORELL, W KROP, S, HITCHCOCK, P, AND RIKER, W F, JR. General systemic actions of di isopropyl fluorophosphate (DFP) in cats. J Pharmacol. & Exper Therap, 87 400-413, 1946
- 229 MORISON, R. S., AND ROSENBLUTH, A. The action of eserine and prostigmin on skeletal muscles Science, 84: 551-552, 1935.
- 230 NACHMANSOHN D Sur l'action de la strychnine. Compt. rend. Soc. de biol., 129: 941-943 1933.
- 231 NACHMANSOHN, D Sur l'inhibition de la cholinesterase. Compt. rend. Soc. de biol., 130: 1045-1048, 1939
- 232 NACHMANSOHN, D.. Role of acetylcholine in mechanism of nerve activity Vitamins and Hormones 3: 337-377, 1945
- 233 NACHMANSOHN, D Chemical mechanism of nerve activity Ann New York Acad. Sci., 47 395-425, 1946.
- 234 NACHMANSOHN D.. Role of acetylcholine in conduction. Bull. Johns Hopkins Hosp 83 463-493, 1948.
- 235 NACHMANSOHN D, AND AUGUSTINSON, K. B Personal Communication.
- 236 NACHMANSOHN D AND FELD E. A. Studies on cholinesterase III. On the mechanism of diisopropyl fluorophosphate action in vivo J Biol. Chem., 171: 715-724, 1947
- 237 NACHMANSOHN D, AND ROTHENBERG, M A. On the specificity of cholinesterase in nervous tissue Science, 100: 454-455 1944.
- 238 NACHMANSOHN, D AND ROTHENBERG M. A. Chemical aspects of the transmission of nerve impulses. Progr Neurol. Psychiat., 1. 59-75 1946.
- 239 NACHMANSOHN D, AND ROTHENBERG M. A. Studies on cholinesterase. I. On the specificity of the enzyme in nerve tissue. J Biol. Chem., 158. 653-666 1945
- 240 NACHMANSOHN D ROTHENBERG, M. A. AND FELD E. A. The in vitro reversibility of cholinesterase inhibition by diisopropylfluorophosphate. Arch. Biochem., 14. 197-211 1947
- 241 NACHMANSOHN D ROTHENBERG, M. A., AND FELD E. A. Studies on cholinesterase. V Kinetics of enzyme inhibition. J Biol. Chem., 174: 247-255, 1948.

- 166 KENSLEY, C J Anti-curare action of certain azo dyes in the frog Fed. Proc., 6 344, 1947
- 167 KIBIAKOW, A W Über humorale Übertragung der Erregung von einem Neuron auf das andere. Arch. f. d. ges. Physiol., 232 432-443, 1933
- 168 KNIGHT, G C Relation of extrinsic nerves to functional activity of the oesophagus Brit J Surg, 22:155-163, 1934
- 169 KOELLE, G B Protection of cholinesterase against irreversible inactivation by DFP in vitro J Pharmacol. & Exper Therap, 88 232-237, 1946
- 170 KOELLE, G B, AND GILMAN, A The relationship between cholinesterase inhibition and the pharmacological action of DFP J Pharmacol. & Exper Therap, 87: 421-434, 1946
- 171 KOELLE, G B, AND GILMAN, A. The chronic toxicity of DFP in dogs, monkeys and rats. J Pharmacol. & Exper Therap, 87 435-448, 1946
- 172 KOELLE, G B, AND KOELLE, E S Serum potassium in dogs unchanged after DFP Unpublished observations.
- 173 KOPFANT, T, DILLE, J M, AND LINEGAR, C R Studies on synergism and antagonism of drugs, the action of physostigmine on autonomic ganglia. J Pharmacol. & Exper Therap, 58 105-110, 1936
- 174 KOPFANT, T, KARCZAK, A G, AND KING, T O Effect of tetraethylpyrophosphate on sympathetic ganglionic activity Science, 1947, 106 492-493
- 175 KOSTER, R Synergisms and antagonisms between physostigmine and di isopropyl fluorophosphate in cats. J Pharmacol. & Exper Therap, 88 39-46, 1946
- 176 KHAYER, O, GOLDSTEIN, A, AND PRACHT, F L Studies on physostigmine and related substances. I Quantitative relationship between dosage of physostigmine and inhibition of cholinesterase activity in blood serum of dogs J Pharmacol. & Exper Therap, 80: 8-30, 1944
- 177 KNOR, S The effect of DFP, di isopropylchlorophosphate, di isopropylphosphite and di-isopropylphosphate on the mechanical response of striated frog muscle Fed Proc, 6: 347, 1947
- 178 KUFFLER, S W Electric potential changes at an isolated nerve-muscle junction J Neurophysiol, 5: 18-24, 1942
- 179 KUFFLER, S W Specific excitability of the endplate region in normal and denervated muscle J Neurophysiol, 6: 99-110, 1943
- 179-A KUFFLER, S W Physiology of neuro-muscular junctions electrical aspects. Fed. Proc., 7: 437-446, 1948
- 180 KULL, M P Prostigmin in the treatment of glaucoma Am J Ophth, 26 57-62, 1943
- 181 LANARI, A Miasthenia gravis y transmisión química neuromuscular Rev Soc argent. de biol, 13: 259, 1937
- 182 LANGLEY, J N AND KATO, T The physiological action of physostigmine and its action on denervated skeletal muscle. J Physiol, 49 410-431 1915
- 183 LAQUEUR, L. Ueber Atropin und Physostigmin und ihre Wirkung auf den intraocularen Druck (Ein Beitrag zur Therapie des Glaucoma.) Arch f Ophth, 23 (part 3) 149-175, 1877
- 184 LAQUEUR, L AND WIGERT, C Beiträge zur Lehre von der Erb'schen Krankheit ueber die Erb'sche Krankheit. Neural Zbl, 20: 594-601, 1901
- 185 LARRABEE, M G AND BRONK, D W Prolonged facilitation of synaptic excitation in sympathetic ganglia. J Neurophysiol, 10: 139-154, 1947
- 186 DELAUBENFELD, M W Cholinesterase Science, 58: 450, 1943
- 187 LAURENT, L P E AND WALKER, M B Oral and parenteral administration of prostigmine and its analogues in myasthenia gravis Lancet, 1 1457-1459, 1936
- 188 LAURENT, L P E, AND WALTREY, W W Influence of large doses of potassium chloride on myasthenia gravis Lancet, 1 1434-1435, 1936
- 189 LEHMANN, G Gastric cardiospasm in the dog Am J Physiol, 143 163-168, 1945
- 190 LEOPOLD, I H, AND COMBON, J H, JR. Effect of neostigmine (prostigmin) and physostigmin upon the denervated iris of the cat Proc Soc Exper Biol & Med, 66:382-384 1945
- 191 LEOPOLD, I H, AND COMBON, J H, JR. Use of di isopropylfluorophosphate ("DFP") in treatment of glaucoma Arch. Ophthal, 36: 1-16, 1946
- 192 LEOPOLD, I H, AND COMBON, J H, JR Effect of DFP on the normal eye Arch Ophthal, 36 17-33, 1946
- 193 LIEVRE, J A Peut-on tenter un traitement chirurgical de la myasthénie Presse méd., 44 991-992, 1936
- 194 LILIENTHAL, J L, JR, AND HARVEY, A M Personal Communication
- 195 LINDEWEAVER, H, AND BURK, D The determination of enzyme dissociation constants J Am Chem Soc., 56 658-668, 1934
- 196 LOEWI, O, AND NAVAZIL, E Über humorale Übertragbarkeit der Herznervenwirkung, über das Schicksal des Vagusstoffes Arch f d ges Physiol 214: 678-688, 1926
- 197 LORENTE DE NÓ, R Liberation of acetylcholine by the superior cervical sympathetic ganglion and the nodose ganglion of the vagus Am. J Physiol 121 331-343, 1938
- 198 LORENTE DE NÓ, R Release of acetylcholine by sympathetic ganglia and synaptic transmission Science, 91: 501-503, 1940
- 199 LORENTE DE NÓ, R Effects of choline and acetylcholine chloride on peripheral nerve fibers J Cell & Comp. Physiol 24 85-97, 1944
- 200 MACINTOSH, F C Liberation of acetylcholine by the perfused superior cervical ganglion. J Physiol., 94: 155-169, 1938
- 201 MACINTOSH, F C Distribution of acetylcholine in peripheral and central nervous systems J Physiol., 99: 436-442, 1941

- 279 STARR, F J, AND RICKETTS, H. T. A note on the in vitro respiration of muscle in myasthenia gravis. *J Lab Clin. Med.*, 30: 532-534 1945
- 280 STAVRAKY, G W. Some aspects of the effects of intravenous injections of acetylcholine on the central nervous system. *Trans. Roy Soc. Canada, Sect. 5*, 37: 127-139, 1943
- 281 STAVRAKY, G W, AND DRAKE, C G. An extension of the "Law of Denervation" to afferent neurones. *Fed. Proc.*, 6: 211 1947
- 282 STEDMAN, E. Studies on the relationship between chemical constitution and physiological action. I. Position isomerism in relation to miotic activity of synthetic urethanes. *Biochem J.*, 20: 719-734, 1926
- 283 STEDMAN, E. Studies on the relationship between chemical constitution and physiological action. II. The miotic activity of urethanes derived from the isomeric hydroxybenzylidimethylamines (HBDM). *Biochem. J.*, 23: 17-24, 1929
- 284 STEDMAN, E. Chemical constitution and miotic action. *Am. J. Physiol.*, 99: 523-529, 1929
- 285 STEDMAN, E. AND BARBER, G. Physostigmine ( eserine ). Part III. *J Chem Soc.* 127: 247-258 1925
- 286 STEDMAN, E., AND STEDMAN, E. Studies on relationship between chemical constitution and physiological action. III. The inhibitory action of certain synthetic urethanes on the activity of liver esterase. *Biochem. J.*, 25: 1147-1167, 1931
- 287 STEDMAN, E., AND STEDMAN, E. Studies on relationship between chemical constitution and physiological action. IV. The inhibitory activity of certain synthetic urethanes on the activity of esterase. *Biochem. J.*, 26: 1214-1222 1932
- 288 STEDMAN, E. STEDMAN, E. AND EASON, L. H. Choline-esterase. An enzyme present in the blood serum of the horse. *Biochem. J.* 26: 2056-2066 1932
- 289 STEWART, G N AND RIDGWAY, J M. The action of drugs upon the output of epinephrin from the adrenals. VII. Physostigmine. *J Pharmacol & Exper Therap.*, 17: 227-248 1921
- 290 STOKKE, H C, AND MORFETH, E. Nature of myasthenia gravis. *Science*, 99: 496-497, 1944.
- 291 STOKKE, H C AND MORFETH, E. Cholinesterase activity of skeletal muscle in various conditions. *Proc. Soc. Exper Biol & Med.* 57: 154-159 1944
- 292 STRATMANN, E L, WEIDIT, R D AND REID, G. Accommodation of nerve in myasthenia gravis and in partial curarization. *Australian J. Exper Biol & Med. Sc.* 24: 313-318 1946
- 293 STRAUSS, O H, AND GOLDSTEIN, A. Zone behavior of enzymes illustrated by the effect of dissociation constant and dilution effect on the system cholinesterase-physostigmine. *J Gen Physiol* 26: 559-585 1943
- 294 TEASDALL, R D AND STAVRAKY, G W. Response of deafferented neurones of the spinal cord to impulses reaching them from higher levels of the central nervous system. *Fed Proc* 7: 123 1948.
- 295 THOMPSON, R H S. The action of chemical vesicants on cholinesterase. *J Physiol*, 105: 370-381 1946
- 296 THOMPSON, V, AND TICE, A. Action of beneficial drugs in myasthenia gravis. Effect of prostigmine and guanidine on serum and muscle potassium. *J Pharmacol & Exper Therap* 73: 455-462 1941
- 297 TOMAN, J E P, WOODBURY, J W, AND WOODBURY, L A. Mechanism of nerve conduction block produced by anti-cholinesterases. *J Neurophysiol* 10: 429-441 1947
- 298 TORDA, C, AND WOLFF, H G. Effect of ether extract of the thymus and pancreas on synthesis of acetylcholine. *Proc. Soc. Exper Biol. & Med.* 57: 69-72, 1944
- 299 TORDA, C, AND WOLFF, H G. Effect of serum and its fractions on acetylcholine synthesis. *Fed. Proc.*, 5: 106, 1946
- 300 UNNA, K. R, AND KIMURA, K. K. Antagonism between curare and physostigmine. *Fed Proc.* 6: 378 1947
- 301 VIETS, H. R. Present status of treatment in myasthenia gravis. *Arch Neurol & Psychiat.*, 47: 559-562, 1942.
- 302 VIETS, H. R. Myasthenia gravis. *J A. M. A.*, 127: 1052-1055, 1945
- 303 VIETS, H. R. Diagnosis of myasthenia gravis in patients with dysphagia. *J A M A.*, 134: 987-992 1947
- 304 VIETS, H. R. AND SCHWAB, R. S. Prostigmine in the diagnosis of myasthenia gravis. *New England J Med.*, 213: 1250-1283 1935
- 305 VIVIND, A. E., AND KOFFANT, T. The interaction between neostigmine and epinephrine and the dimethyl-piperidines. *Fed Proc.*, 5: 209, 1946
- 306 VON SALLMANN, L. AND DILLON, B. The effect of DFP on the capillaries of the anterior segment of the eye in rabbits. *Am J Ophth* 30: 1244-1263 1947
- 307 VON SALLMANN, L. AND STONE, W. C. Personal Communication.
- 308 WALDECH, H. AND NACHMANSOHN, D. On the toxicity of atabrine. *Proc. Soc. Exper Biol. & Med.*, 54: 336-338, 1943
- 309 WALKER, M. B. Treatment of myasthenia gravis with physostigmine. *Lancet* 1: 1200-1201 1934.
- 310 WALKER, M. B. Case showing the effect of prostigmine on myasthenia gravis. *Proc Roy Soc. Med.*, 28: 752-761, 1935
- 311 WALKER, M. B. Myasthenia gravis: a case in which fatigue of forearm muscles could induce paralysis of extraocular muscles. *Proc Roy Soc. Med.* 31: 722, 1938.
- 312 WELSH, J. H., AND HYDE, J. E. The thymus and acetylcholine synthesis. *Science* 102: 252-253 1945
- 313 WESCOE, W. C. GREEN, R. E., McNAMARA, B. P., AND KRUP, S. The influence of atropine and scopolamine on the central effects of DFP. *J Pharmacol & Exper Therap.*, 92: 63-72, 1948.
- 314 WESCOE, W. C., HUNT, C. C., RIKER, W. F., AND LITT, I. C. Regeneration rates of serum cholinesterase in normal individuals and in patients with liver damage. *Am J Physiol* 149: 549-551, 1947
- 315 WHITE, A. C. AND STROMAN, E. On the physostigmine-like action of certain synthetic urethanes. *J Pharmacol & Exper Therap* 41: 229-233, 1931

GEORGE B KOELLE AND ALFRED GILMAN

- 242 NACHMANSOHN, D., AND SCHNEEMANN, H. On the effect of drugs on cholinesterase. *J Biol Chem.*, 159: 239-240, 1945
- 243 ODOM, G., RUSSEL C K., AND McEACHERN, D. The myogram, blood cholinesterase, and effect of prostigmine in myasthenia gravis and progressive muscular atrophy. *Brain*, 86: 1-17, 1943
- 244 OPPENHEIM, H. Die myasthenische Paralyse (Bulboparalyse ohne anatomischen Befund). *S Karger, Berlin*, 1901
- 245 PICKFORD, M. The action of acetylcholine in the supraoptic nucleus of the chloralosed dog. *J Physiol*, 106: 264-270, 1947
- 246 POER, D. H. Effect of removal of malignant thymic tumor in a case of myasthenia gravis. *Am J Surg*, 115: 553-595, 1942
- 247 PRITCHARD, E. A. B. The use of "prostigmin" in the treatment of myasthenia gravis. *Lancet* 1: 432-434, 1945
- 248 QUILLIAM, J. P., AND STROGO, F. G. The action of DFP on the isolated rabbit heart preparation. *J Physiol*, 106: 23P, 1947
- 249 RAVENTOS, J. Pharmacological actions of quaternary ammonium salts. *Quart J Exper Physiol*, 26: 361-374, 1937
- 250 RAVENTOS, J. Synergisms and antagonisms of acetylcholine by quaternary ammonium salts. *Quart J Exper Physiol*, 27: 99-111, 1938
- 251 REANDOV, M. J., MARZONI, F. A., AND HENDRIX, J. P. The effect of neostigmine (Prostigmine) on the actions of tetraethylammonium (Etamon) in dogs and man. *Fed Proc*, 6: 364, 1947
- 252 RENTZ, E. Methylene blue and cholinesterase. *Arch f exptl Path u Pharmacol* 196: 148-160, 1940
- 253 RICHTER, D. AND CHOFT, P. C. Blood esterase. *Biochem J* 36: 748-757, 1942
- 254 RIKER, W. F. JR., AND WESCOX, W. C. The direct action of prostigmine on skeletal muscle, its relationship to the choline esters. *J Pharmacol & Exper Therap*, 83: 58-68, 1946
- 255 ROBIN, F. H. The history of physostigmine in the practice of ophthalmology. *Am J Ophth* 30: 19-23, 1947
- 256 ROEDER, K. D., KENNEDY, N. L., AND SAMSON, E. A. Synaptic conduction to giant fibers of the cockroach and the action of anti cholinesterases. *J Neurophysiol*, 10: 1-10, 1947
- 257 RY, M. H. Study of cholinesterases. *J Pharmacol & Exper Therap*, 59: 264-270, 1937
- 258 ENBLUETH, A. AND CANNON, W. B. Effects of preganglionic denervation on the superior cervical ganglion. *Fed Proc.*, 5: 189, 1946.
- 259 ENBLUETH, A. AND LUCO, J. V. A study of denervated mammalian skeletal muscle. *Am. J Physiol.* 122: 688-707, 1938
- 260 ENBLUETH, A. AND SIMONE, F. A. Responses of superior cervical ganglion to single and repetitive stimuli. *J Physiol*, 122: 708-721, 1938
- 261 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 262 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 263 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 264 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 265 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 266 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 267 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 268 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 269 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 270 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 271 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 272 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 273 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 274 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 275 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 276 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 277 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 278 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 279 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 280 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 281 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 282 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 283 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 284 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 285 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 286 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 287 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 288 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 289 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 290 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 291 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 292 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 293 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 294 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 295 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 296 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 297 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 298 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 299 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948
- 300 ENBLUETH, A. AND SPRINSON, D. B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol*, 116, 1948



27. STARR, F. J., AND RICKETTS, H. T. A note on the in vitro respiration of muscle in myasthenia gravis. *J. Lab. Clin. Med.*, 30: 552-554, 1945
28. STAVRAKY, G. W. Some aspects of the effects of intravenous injections of acetylcholine on the central nervous system. *Trans. Roy. Soc. Canada, Sect. 5*, 37: 127-139, 1943
29. STAVRAKY, G. W., AND DRAKE, C. G. An extension of the "Law of Denervation" to afferent neurones. *Fed. Proc.*, 4: 211, 1947
30. STEEDMAN, E. Studies on the relationship between chemical constitution and physiological action. I. Position isomerism in relation to miotic activity of synthetic urethanes. *Biochem. J.* 23: 719-734, 1928
31. STEEDMAN, E. Studies on the relationship between chemical constitution and physiological action. II. The miotic activity of urethanes derived from the isomeric hydroxybenzylidimethylamines (HBDM). *Biochem. J.*, 23: 17-24, 1929
32. STEEDMAN, E. Chemical constitution and miotic action. *Am. J. Physiol.* 93: 523-529, 1929
33. STEEDMAN, E., AND BARNER, G. Physostigmine ( eserine ). Part III. *J. Chem. Soc.* 127: 247-258, 1925
34. STEEDMAN, E., AND STEEDMAN, E. Studies on relationship between chemical constitution and physiological action. III. The inhibitory action of certain synthetic urethanes on the activity of liver esterase. *Biochem. J.*, 25: 1147-1167, 1931
35. STEEDMAN, E., AND STEEDMAN, E. Studies on relationship between chemical constitution and physiological action. IV. The inhibitory activity of certain synthetic urethanes on the activity of esterase. *Biochem. J.* 26: 1214-1222, 1932
36. STEEDMAN, E., STEEDMAN, E., AND EASON, L. H. Choline-esterase. An enzyme present in the blood serum of the horse. *Biochem. J.* 26: 2056-2066, 1932
37. STEWART, G. N., AND ROGOFF, J. M. The action of drugs upon the output of epinephrine from the adrenals. VII. Physostigmine. *J. Pharmacol. & Exper. Therap.* 17: 227-248, 1921
38. STOKER, H. C., AND MORFETH, E. Nature of myasthenia gravis. *Science*, 99: 495-497, 1944
39. STOKER, H. C., AND MORFETH, E. Cholinesterase activity of skeletal muscle in various conditions. *Proc. Soc. Exper. Biol. & Med.*, 57: 154-159, 1944
40. STRATMANN, E. L., WRIGHT, R. D., AND REID, G. Accommodation of nerve in myasthenia gravis and in partial curarization. *Australian J. Exper. Biol. & Med. Sc.* 24: 313-318, 1945
41. STRAUS, O. H., AND GOLDSTEIN, A. Zone behavior of enzymes illustrated by the effect of dissociation constant and dilution effect on the system cholinesterase-physostigmine. *J. Gen. Physiol.* 26: 559-585, 1943
42. TEASDALE, R. D., AND STAVRAKY, G. W. Response of deafferented neurones of the spinal cord to impulses reaching them from higher levels of the central nervous system. *Fed. Proc.*, 7: 123, 1948
43. THOMPSON, R. H. S. The action of chemical vesicants on cholinesterase. *J. Physiol.*, 105: 370-381, 1946
44. THOMPSON, V., AND TICE, A. Action of beneficial drugs in myasthenia gravis. Effect of physostigmine and guanidine on serum and muscle potassium. *J. Pharmacol. & Exper. Therap.* 73: 455-462, 1941
45. TOMAN, J. E. P., WOODBURY, J. W., AND WOODBURY, L. A. Mechanism of nerve conduction block produced by anti-cholinesterases. *J. Neurophysiol.* 10: 423-441, 1947
46. TORDA, C., AND WOLFF, H. G. Effect of ether extract of the thymus and pancreas on synthesis of acetylcholine. *Proc. Soc. Exper. Biol. & Med.* 57: 62-72, 1944
47. TORDA, C., AND WOLFF, H. G. Effect of serum and its fractions on acetylcholine synthesis. *Fed. Proc.* 5: 106, 1946
48. UNNA, K. R., AND HIMURA, K. K. Antagonism between curare and physostigmine. *Fed. Proc.* 6: 378, 1947
49. VIETS, H. R. Present status of treatment in myasthenia gravis. *Arch. Neurol. & Psychiat.*, 47: 559-562, 1942
50. VIETS, H. R. Myasthenia gravis. *J. A. M. A.* 127: 1053-1056, 1945
51. VIETS, H. R. Diagnosis of myasthenia gravis in patients with dysphagia. *J. A. M. A.*, 134: 987-992, 1947
52. VIETS, H. R., AND SCHWAB, R. S. Physostigmine in the diagnosis of myasthenia gravis. *New England J. Med.*, 213: 1280-1283, 1935
53. VIVINO, A. E., AND KOFFANY, T. The interaction between neostigmine and epinephrine and the dimethyl piperidines. *Fed. Proc.* 5: 209, 1946
54. VON SALLMANN, L., AND DILLON, B. The effect of DFP on the capillaries of the anterior segment of the eye in rabbits. *Am. J. Ophth.* 30: 1244-1252, 1947
55. VON SALLMANN, L., AND STONE, W. C. Personal Communication
56. WACHS, H., AND NACHMANSON, D. On the toxicity of atabrine. *Proc. Soc. Exper. Biol. & Med.*, 54: 336-338, 1943
57. WALKER, M. B. Treatment of myasthenia gravis with physostigmine. *Lancet* 1: 1200-1201, 1934
58. WALKER, M. B. Case showing the effect of physostigmine on myasthenia gravis. *Proc. Roy. Soc. Med.*, 28: 752-761, 1935
59. WALKER, M. B. Myasthenia gravis a case in which fatigue of forearm muscles could induce paralysis of extraocular muscles. *Proc. Roy. Soc. Med.* 31: 722, 1938
60. WELSH, J. H., AND HYDE, J. E. The thymus and acetylcholine synthesis. *Science*, 102: 252-253, 1945
61. WESCOE, W. C., GREEN, R. E., McNAMARA, B. P., AND KROP, S. The influence of atropine and scopolamine on the central effects of DFP. *J. Pharmacol. & Exper. Therap.* 92: 63-72, 1948
62. WESCOE, W. C., HUNT, C. C., RIKER, W. F., AND LITT, I. C. Regeneration rates of serum cholinesterase in normal individuals and in patients with liver damage. *Am. J. Physiol.* 149: 549-551, 1947
63. WHITE, A. C., AND STEEDMAN, E. On the physostigmine like action of certain synthetic urethanes. *J. Pharmacol. & Exper. Therap.* 41: 259-268, 1931

- 242 NACHMANSOHN, D., AND SCHNEEMANN, H. On the effect of drugs on cholinesterase. *J Biol Chem.*, 159: 239-240, 1945
- 243 ODOM, G., RUSSEL, C K., AND McEACHERN, D. The myogram, blood cholinesterase, and effect of prostigmine in myasthenia gravis and progressive muscular atrophy. *Brain*, 86: 1-17, 1943
- 244 OFFENHEIM, H. Die myasthenische Paralyse (Bulboparalyse ohne anatomischen Befund). *B Karger, Berlin*, 1901
- 245 PICKFORD, M. The action of acetylcholine in the supraoptic nucleus of the chloralosed dog. *J Physiol.*, 104: 264-270, 1947
- 246 POER, D H. Effect of removal of malignant thymic tumor in a case of myasthenia gravis. *Am J Surg.*, 115: 588-595, 1942
- 247 PRITCHARD, E A B. The use of "prostigmin" in the treatment of myasthenia gravis. *Lancet*, 1: 432-434, 1933
- 248 QUILLIAN, J P., AND STRONG, F G. The action of DFP on the isolated rabbit heart preparation. *J Physiol.*, 106: 23P, 1947
- 249 RAVENHOS, J. Pharmacological actions of quaternary ammonium salts. *Quart J Exper Physiol.*, 24: 361-374, 1937
- 250 RAVENHOS, J. Synergisms and antagonisms of acetylcholine by quaternary ammonium salts. *Quart. J Exper Physiol.*, 27: 99-111, 1938
- 251 REARDON, M J., MARZONI, F A., AND HENDEIX, J P. The effect of neostigmine (Prostigmine) on the actions of tetraethylammonium (Etamon) in dogs and man. *Fed Proc.*, 6: 364, 1947
- 252 RENTS, E. Methylene blue and cholinesterase. *Arch f exptl Path u Pharmacol.*, 196: 145-160, 1940
- 253 RICHTER, D., AND CROFT, P C. Blood esterases. *Biochem J.*, 34: 748-757, 1942
- 254 RIKER, W F, JR., AND WESCOE, W C. The direct action of prostigmine on skeletal muscle, its relationship to the choline esters. *J Pharmacol & Exper Therap.*, 88: 58-65, 1946
- 255 ROTH, K H. The history of physostigmine in the practice of ophthalmology. *Am J Ophth.*, 30: 19-24, 1947
- 256 ROEDER, K D., KENNEDY, N K., AND SAMSON, E A. Synaptic conduction to giant fibers of the cockroach and the action of anti cholinesterases. *J Neurophysiol.*, 10: 1-10, 1947
- 257 ROEFKE, M H. Study of cholinesterase. *J Pharmacol & Exper Therap.*, 59: 264-276, 1937
- 258 ROOT, M A. Dose and intensity of action and elimination of prostigmine. *Fed Proc.*, 5: 199, 1946
- 259 ROSENBLUTH, A., AND CANNON, W B. Effects of preganglionic denervation on the superior cervical ganglion. *Am J Physiol.*, 125: 276-283, 1939
- 260 ROSENBLUTH, A., AND LECO, J V. A study of denervated mammalian skeletal muscle. *Am J Physiol.*, 120: 781-797, 1937
- 261 ROSENBLUTH, A., AND SIMEONE, F A. Responses of superior cervical ganglion to single and repetitive activation. *Am J Physiol.*, 122: 688-707, 1938
- 262 ROSENBLUTH, A., AND SIMEONE, F A. The action of eserine or prostigmin on the superior cervical ganglion. *Am J Physiol.*, 122: 708-721, 1938
- 263 ROTHENBERG, M A., SPRINSON, D B., AND NACHMANSOHN, D. Site of action of acetylcholine. *J Neurophysiol.*, 11: 111-116, 1948
- 264 SAWYER, C H. Hydrolysis of choline esters by liver. *Science*, 101: 385-388, 1945
- 265 SCHALLER, W., AND WEINBAUM, C A G. The influence of various drugs on a crustacean synapse. *J Cell. Physiol.*, 31: 35-47, 1948
- 266 SCHÄR WÖRTHICH, B. Die Wirkungen einiger kreislaufaktiver Substanzen auf Fermente. *Helv. chim. acta.*, 26: 1836-1856, 1943
- 267 SCHLEZINGER, N S. Evaluation of therapy in myasthenia gravis. *Arch Int Med.*, 65: 60-77, 1940
- 268 SCHOLE, R O. Studies on the ocular reactions of rabbits to di isopropyl fluorophosphate. *J Pharmacol. & Exper Therap.*, 88: 23-26, 1946
- 269 SCHOLE, R O., AND WALLAN, L J. The effect of di isopropyl fluorophosphate on normal human eyes. *J Pharmacol & Exper Therap.*, 88: 238-245, 1946
- 270 SCHWEITZER, A., STEEDMAN, E., AND WRIGHT, S. Central action of anticholinesterases. *J Physiol.*, 96: 302-336, 1939
- 271 SECKER, J. The humoral control of the secretion by the submaxillary gland of the cat following chorda stimulation. *J Physiol.*, 81: 81-92, 1934
- 272 SJOSTRAND, T. Potential changes in the cerebral cortex of the rabbit arising from cellular activity and the transmission of impulses in the white matter. *J Physiol.*, 90: 41P-43P, 1937
- 273 SLAUGHTER, D. The modifying action of neostigmine on pain threshold responses to various opiates. *Fed. Proc.*, 5: 202, 1946
- 274 SLAUGHTER, D., PARSONS, J C., AND MUNAL, H D. New clinical aspects of analgesic action of morphine. *J A M A*, 115: 2558-2560, 1940
- 275 SMITH, M I., ELYSE, E., AND FRANKER, W H. The pharmacological actions of certain phenol esters, with special reference to the etiology of so-called ginger paralysis. *U S Pub Health Rep.*, 45: 2509-2524, 1930
- 276 SMITH, W K. The differential action of erythroidine in the normal and in the decerebrate animal. *Fed Proc.*, 6: 205, 1947
- 277 SOBOTKA, H., AND ANTROPOL, W. Inhibitors of cholinesterase. *Enzymologia*, 4: 189-191, 1937
- 278 SPERLING, F., KARCZMAR, A G., KING, T O., AND KOFFANYI, T. The effects of DFP and physostigmine on the sympathetic ganglia. *Fed Proc.*, 6: 373, 1947

- 279 STARR, F J, AND RICKETTS, H T A note on the in vitro respiration of muscle in myasthenia gravis. J Lab Clin. Med., 30: 582-594, 1945
- 280 STAVRAKY, G W Some aspects of the effects of intravenous injections of acetylcholine on the central nervous system. Trans. Roy Soc. Canada, Sect 5, 37 127-132, 1943
281. STAVRAKY, G W, AND DRAKE, C G An extension of the "Law of Denervation" to afferent neurones. Fed. Proc., 6 211 1947
282. STEDMAN, E. Studies on the relationship between chemical constitution and physiological action. I. Position isomerism in relation to miotic activity of synthetic urethanes. Biochem J, 20 719-734 1928
283. STEDMAN, E. Studies on the relationship between chemical constitution and physiological action. II The miotic activity of urethanes derived from the isomeric hydroxybenzylidimethylamines (HBDM) Biochem. J, 23 17-24, 1929
- 284 STEDMAN, E Chemical constitution and miotic action. Am. J Physiol. 90: 528-529 1929
- 285 STEDMAN, E., AND BARBER, G Physostigmine (eserine) Part III. J Chem. Soc., 127 247-258, 1925
286. STEDMAN, E., AND STEDMAN, E Studies on relationship between chemical constitution and physiological action. III The inhibitory action of certain synthetic urethanes on the activity of liver esterase. Biochem J, 25: 1147-1167, 1931
- 287 STEDMAN, E. AND STEDMAN, E. Studies on relationship between chemical constitution and physiological action. IV The inhibitory activity of certain synthetic urethanes on the activity of esterases Biochem. J 26 1214-1222, 1932.
288. STEDMAN, E., STEDMAN, E. AND EASON, L. H Choline-esterase. An enzyme present in the blood serum of the horse Biochem. J 26 2056-2058, 1932
- 289 STEWART, G N AND ROGOFF, J M The action of drugs upon the output of epinephrin from the adrenals. VII. Physostigmine J Pharmacol & Exper Therap 17 227-248, 1921
- 290 STOKER, H C AND MORFETH E Nature of myasthenia gravis Science, 99: 495-497, 1944
- 291 STOKER H C AND MORFETH E Cholinesterase activity of skeletal muscle in various conditions. Proc Soc. Exper Biol & Med., 57 154-159 1944.
292. STRATMANN E L, WRIGHT R D, AND REIG G Accomodation of nerve in myasthenia gravis and in partial curarization Australas J Exper Biol & Med Sc., 24. 313-318 1945
- 293 STRAUS, O H, AND GOLDSTEIN A Zone behavior of enzymes illustrated by the effect of dissociation constant and dilution effect on the system cholinesterase-physostigmine J Gen Physiol 26 559-585 1943
294. TEASDALE, R D AND STAVRAKY G W Response of desferented neurones of the spinal cord to impulses reaching them from higher levels of the central nervous system Fed Proc. 7: 123 1948.
295. THOMPSON, R. H S The action of chemical vesicants on cholinesterase J Physiol 105 370-381 1946.
296. THOMPSON V AND TICE A Action of beneficial drugs in myasthenia gravis Effect of prostigmine and guanidine on serum and muscle potassium. J Pharmacol & Exper Therap 73 455-462 1941
- 297 TOMAN, J E P, WOODBURY J W, AND WOODBURY, L A. Mechanism of nerve conduction block produced by anti-cholinesterases J Neurophysiol., 10 429-441 1947
298. TORDA, C AND WOLFF H G Effect of ether extract of the thymus and pancreas on synthesis of acetylcholine Proc. Soc Exper Biol. & Med 57 69-72 1944
- 299 TORDA, C AND WOLFF H G Effect of serum and its fractions on acetylcholine synthesis. Fed. Proc. 5 106, 1946
- 300 UNNA K R AND KIMURA K. K. Antagonism between curare and physostigmine. Fed Proc. 6 378 1947
- 301 VIETS, H R Present status of treatment in myasthenia gravis. Arch Neurol & Psychiat., 47: 859-862, 1942.
- 302 VIETS, H R Myasthenia gravis J A. M A 127 1082-1098 1945
- 303 VIETS, H R Diagnosis of myasthenia gravis in patients with dysphagia J A M A, 134 987-992 1947
304. VIETS H R AND SCHWAB R. S Prostigmine in the diagnosis of myasthenia gravis. New England J Med., 213 1280-1283 1935
- 305 VIVINO, A. E. AND KOPFANT T The interaction between neostigmine and epinephrine and the dimethyl piperidines Fed Proc , 5 209, 1946
- 306 VON SALLMANN L AND DILON B The effect of DFP on the capillaries of the anterior segment of the eye in rabbits Am J Ophth. 30: 1244-1262 1947
- 307 VON SALLMANN L AND STONE, W C Personal Communication.
308. WAELSCH H AND NACHMANSOHN, D On the toxicity of atabrine Proc. Soc. Exper Biol. & Med , 54. 336-338, 1943
- 309 WALKER, M B Treatment of myasthenia gravis with physostigmine. Lancet 1 1200-1201 1934
- 310 WALKER M B Case showing the effect of prostigmine on myasthenia gravis Proc Roy Soc. Med , 28: 759-761 1935.
- 311 WALKER, M B Myasthenia gravis a case in which fatigue of forearm muscles could induce paralysis of extraocular muscles Proc. Roy Soc. Med, 31 722, 1938.
312. WELSH J H, AND HYDE J E. The thymus and acetylcholine synthesis Science, 102 252-253 1945
- 313 WESCOE, W C GREEN, R. E. McNAMARA, B P, AND KROP S The influence of atropine and scopolamine on the central effects of DFP J Pharmacol & Exper Therap 92 63-72, 1948.
314. WESCOE, W C HUNT C C RIKER, W F AND LITT I C Regeneration rates of serum cholinesterase in normal individuals and in patients with liver damage. Am J Physiol 149 549-551, 1947
- 315 WHITE A C AND STEDMAN E On the physostigmine-like action of certain synthetic urethanes. J Pharmacol & Exper Therap 41: 259-268, 1931

GEORGE B KOELLE AND ALFRED GILMAN

316. WIKLER, A Effects of morphine, nembutal, ether and eserine on two-neuron and multi neuron reflexes in the cat Proc Soc Exper Biol & Med., 53: 193-199, 1943
317. WILSON, A, AND STONER, H B Myasthenia gravis Consideration of its causation in a study of fourteen cases. Quart. J Med New Series, 13 1-18, 1943
318. WRAMNER, T The potentiating effect of neostigmine and eserine on the action of morphine as measured by the Straub-Hermann reaction Acta physiol Scandinav, 9: 336-340, 1945
319. WRIGHT, C I The inhibition of cholinesterase by aromatic amino alcohols of the type Ar-CHOHCH<sub>2</sub>NH<sub>2</sub>. J Pharmacol & Exper Therap, 87: 109-120, 1943
320. WRIGHT, C I, AND SABINE, J C Cholinesterases of human erythrocytes and plasma and their inhibition by antimalarial drugs J Pharmacol & Exper Therap, 93: 230-239, 1943.
321. WRIGHT, C I, SABINE, J C, AND DORN, H F Inactivation of cholinesterase by morphine, dihydric, codeine and desomorphine J Pharmacol & Exper Therap, 78 375-385, 1943
322. ZELLEN, E A, AND BISSKOPF, A Über die cholin-esterase des gehirns und der erythrocyten Helv. Acta, 26: 1619-1631, 1943

# THE METABOLISM OF BARBITURATES\*

E W MAYNERT AND H B VAN DYKE

*The Department of Pharmacology, College of Physicians and Surgeons Columbia University,  
New York, N Y*

This review is an attempt to examine critically a limited part of the large volume of literature which has appeared on the metabolism of barbiturates. A more general treatment of the pharmacology and therapeutic use of this group of drugs may be found in several other reviews (50, 51, 91, 120, 121). The following discussion is restricted to absorption, distribution in tissues, sites of degradation, excretion and metabolic fate of the drugs. Also, the methods, both chemical and pharmacological, which have been used in studies of the barbiturates are examined. Consideration of excretion and metabolic fate is limited to those drugs which have received clinical use or are closely related to such drugs. Studies of the effect of stimulants, diuretics and possible metabolic inhibitors on the metabolism of the barbiturates are beyond the scope of this paper.

Inasmuch as trade names or U S P names are used for convenience, a glossary and index appear at the end of this review. There the reader will find synonyms, chemical structures and page references. Strict chemical terminology is not followed in the discussion to the extent that "barbituric acid" and "barbiturate" are used interchangeably.

## METHODS

The relative value of all information concerning the distribution and excretion of barbituric acid derivatives depends upon the reliability of the quantitative methods used to obtain this information. For the purpose of discussion the methods may be divided into five types: (1) gravimetric, (2) colorimetric, (3) ultraviolet spectrophotometric, (4) isotopic and (5) pharmacological.

### (1) Gravimetric Methods

This method has probably been used more than any other for the determination of unchanged barbiturate in the urine. It was first used by Fischer and von Mering (33) at the time of the introduction of barbital into medicine. Many modifications have been described, but a common feature of all is the extraction of the barbituric acid from the urine with an organic solvent, usually ether or ethyl acetate (48, 105). After evaporation of the solvent the barbiturate is purified and weighed.

The problem of obtaining the barbituric acid in as pure form as possible without the losses which accompany recrystallization has been attacked in many different ways. Pucher (105) employed a preliminary extraction of the urine with petroleum ether in which the barbiturates are insoluble. Klingenfuss and Reinert (68) made the urine alkaline and carried out a preliminary extraction with ether.

\* This review was made possible by a grant from the United States Public Health Service for investigative work in this field.

Other investigators have chosen to use only one extraction and purify the crude barbiturate after evaporation of the solvent. If the barbituric acid contains only saturated groups, some of the impurities may be removed by oxidation with potassium permanganate (49, 105) or potassium dichromate (113). The barbiturate may be purified by precipitation with mercuric sulfate (36) or as a copper sulfate-pyridine complex (132). These procedures yield pure products, but the loss of barbiturate may be high (68). The crude barbiturate may be treated with charcoal (4) which removes color and often yields a pure product, however, there is always at least a small loss of barbiturate due to adsorption on the charcoal (48, 113). The crude product may also be purified by sublimation. This technic has been successful in many cases (55, 118), especially when the amount of product was small.

The results obtained by the gravimetric method are of doubtful value unless the product is pure enough to allow its identification by melting point and mixed melting point determinations. If the amount of product is large enough, a crystalline derivative of the barbituric acid such as the di-*p*-nitrobenzyl derivative (49) or the dioxanthyl derivative (31, 32) may be prepared for additional proof of identity. The numerous qualitative color and precipitation tests which have been proposed are all of questionable value.

The gravimetric method has been used with considerable success for the determination of barbital in the urine. It is probable that the method can be adapted to yield results of comparable significance for many of the other barbiturates, even though they are administered in much smaller doses and are largely degraded in the body. However, adequate control experiments must be run with each new drug to determine completeness of extraction, loss during purification, and the limits of the method. With drugs which are degraded *in vivo* it becomes even more important than with barbital to determine the identity of the product by the determination of the melting point. Unfortunately, many studies which have appeared on the excretion of barbiturates in the urine contain no data on the adequacy of extraction or the purity of the final product. With drugs which may yield closely related metabolites in the urine it obviously is not desirable to circumvent the final isolation by running Kjeldahl nitrogens (115) or determining barbituric acid derivatives by an argentimetric method (116).

The greatest limitation of the gravimetric method is that a relatively large amount of barbiturate (ca. 5 to 10 mgm) must be present in the sample for an adequate determination. This consideration has made the technic very difficult for studies of the concentration of barbiturates in various tissues under the conditions of usual dosage. Large samples of tissue (100 to 1000 gm) must be employed. Furthermore, the isolation of barbiturates from tissue is much more difficult than from urine, although Herwick (55) and Kozelka (84) have designed good procedures for quantitative recovery. Nevertheless, examination of tissues has usually been limited to cases of fatal poisoning (2, 105).

## (2) Colorimetric Methods

The colorimetric method for barbiturates which has received most study depends upon the interaction of the barbiturates with a cobaltous salt in an alkaline

medium. It was used as a qualitative test by Parri, Zwikker (132) and Bodendorf and was adopted for quantitative purposes by Koppányi (79, 80). The first method described by Koppányi was a visual comparison procedure in which a blue color was generated in a barbiturate-containing chloroform solution by the addition of anhydrous methanolic solutions of cobaltous acetate and barium or lithium hydroxide. The chromophore was unstable and quickly precipitated or faded. Later (73, 81) it was discovered that the use of isopropyl amine in place of the inorganic hydroxide yielded a stable color which could be measured in a standard colorimeter.

There have been several investigations of the specificity of the cobalt color. The test can apparently be used for all the disubstituted barbituric acids and thiobarbituric acids, although no linear relationship exists between the intensity of color and the molecular weight of the barbiturate (8, 21, 27). Koppányi found that acetic acid and other chloroform-soluble acids give color in the test. This has been confirmed by Riley (112) who found that aldehydes also introduce errors. Koppányi noted that theophylline and theobromine give a color similar to the barbituric acids, but he stated that these compounds can never be present in body tissues or fluids in concentrations sufficiently high to give a positive test. Thymine, biuret, guanidine, oxamide, hippuric acid and creatinine also yield color, but most of these compounds apparently do not enter chloroform extracts in detectable amounts. Lecithin and other phospholipids interfere with the color test, apparently by holding the lipophilic barbiturates in loose combination and preventing them from reacting with the cobalt (72). The phospholipids from tissue are removed by treatment with copper sulfate and sodium hydroxide, or they may be precipitated from the chloroform extracts with acetone (90). Sulfonamides give color in the cobaltous acetate-isopropyl amine test, but 100 mgm of sulfonamide give less color than 2 mgm of phenobarbital (82). Riley showed that the substituted acetyl ureas and acetamides which are *in vitro* hydrolytic products of the barbiturates do not yield any color. The cobalt color test is sometimes carried out in alcoholic solution rather than in chloroform, and various alkaline reagents are used. Studies of the specificity of the reaction under these conditions have been reported by Herwick (55), Mohrschulz (97) and Kozelka (85).

A considerable amount of criticism has been directed against the use of the cobalt color for the quantitative determination of barbiturates. Sack (113) reported that the color is very unstable and fades with the appearance of turbidity. Furthermore, he stated that the difference between the "blue" solutions of various barbiturate concentrations is neither large nor constant. Iversen (58) reported that the color of the complex compound is not suitable for colorimetry, since photoelectric tests showed that the color intensity for a given barbiturate concentration is not reproducible and not constant. He felt that an additional drawback was the fact that an approximate proportionality between the color intensity and the concentration was present only within the very narrow range of 20 to 100 mgm per cent barbiturate in chloroform. Kozelka (84) studied the cobalt color in absolute alcohol solution, using sodium ethoxide as the alkaline reagent. He found that the cobalt and sodium must be present in a definite ratio

Other investigators have chosen to use only one extraction and purify the crude barbiturate after evaporation of the solvent. If the barbituric acid contains only saturated groups, some of the impurities may be removed by oxidation with potassium permanganate (49, 105) or potassium dichromate (113). The barbiturate may be purified by precipitation with mercuric sulfate (36) or as a copper sulfate-pyridine complex (132). These procedures yield pure products, but the loss of barbiturate may be high (68). The crude barbiturate may be treated with charcoal (4) which removes color and often yields a pure product, however, there is always at least a small loss of barbiturate due to adsorption on the charcoal (48, 113). The crude product may also be purified by sublimation. This technic has been successful in many cases (55, 118), especially when the amount of product was small.

The results obtained by the gravimetric method are of doubtful value unless the product is pure enough to allow its identification by melting point and mixed melting point determinations. If the amount of product is large enough, a crystalline derivative of the barbituric acid such as the di-*p*-nitrobenzyl derivative (49) or the dioxanthyl derivative (31, 32) may be prepared for additional proof of identity. The numerous qualitative color and precipitation tests which have been proposed are all of questionable value.

The gravimetric method has been used with considerable success for the determination of barbital in the urine. It is probable that the method can be adapted to yield results of comparable significance for many of the other barbiturates, even though they are administered in much smaller doses and are largely degraded in the body. However, adequate control experiments must be run with each new drug to determine completeness of extraction, loss during purification, and the limits of the method. With drugs which are degraded *in vivo* it becomes even more important than with barbital to determine the identity of the product by the determination of the melting point. Unfortunately, many studies which have appeared on the excretion of barbiturates in the urine contain no data on the adequacy of extraction or the purity of the final product. With drugs which may yield closely related metabolites in the urine it obviously is not desirable to circumvent the final isolation by running Kjeldahl nitrogens (115) or determining barbituric acid derivatives by an argentimetric method (116).

The greatest limitation of the gravimetric method is that a relatively large amount of barbiturate (ca. 5 to 10 mgm) must be present in the sample for an adequate determination. This consideration has made the technic very difficult for studies of the concentration of barbiturates in various tissues under the conditions of usual dosage. Large samples of tissue (100 to 1000 gm) must be employed. Furthermore, the isolation of barbiturates from tissue is much more difficult than from urine, although Herwick (55) and Kozelka (84) have designed good procedures for quantitative recovery. Nevertheless, examination of tissues has usually been limited to cases of fatal poisoning (2, 105).

### (2) Colorimetric Methods

The colorimetric method for barbiturates which has received most study depends upon the interaction of the barbiturates with a cobaltous salt in an alkaline



method, Koppanyi (74) found that 5 to 41 per cent of doses of Neonal and about 8 per cent of doses of Amytal are excreted in the urine. In contrast, Herwick (55) and Shonle (118) using chemical isolation and pharmacological methods showed that these substances are excreted only in traces. Likewise, Krause (88) showed that dogs fed Amytal excrete in the urine in 48 hours color-producing substances equivalent to about 16 per cent of the dose. However, the substances gave no precipitate with copper sulfate and pyridine. Using a procedure involving adsorption on charcoal and elution Brundage and Gruber (8) found that Ortol and pentobarbital yield substances which give color with cobalt but do not have appreciable pharmacological activity. Koppanyi (46, 77) has also shown that metabolites of the barbiturates yield color with cobalt.

Certain other colorimetric methods not employing cobalt have been used for the quantitative determination of barbiturates. In her studies on the distribution of Dial and Phanodorn in the central nervous system, Vogt (126) used a method involving titration with potassium permanganate. The method was not stoichiometric, and calibration curves were required. Owing to the extensive purification procedure employed, her recovery of the barbiturates added to tissue was only about 50 per cent. Raventós (108) has used a green color produced by the addition of anhydrous methanolic solutions of copper sulfate and diethyl amine to a chloroform solution of a thiobarbiturate. He demonstrated good recoveries of Kemithal added to blood, liver and brain. The procedures of Vogt and Raventós are subject to the criticism that it has not been proved that some metabolites of the drugs do not react in the same manner as unchanged drug.

### *(3) Ultraviolet Spectrophotometric Methods*

It has been recognized for a number of years that 5,5-dialkylbarbiturates in aqueous alkaline solution have a characteristic absorption in the ultraviolet region of the spectrum. Hellmann (52) was the first to describe a spectrophotometric method for the estimation of thiopental in blood. His procedure utilized a characteristic absorption band at  $288\text{ m}\mu$  in ether solution. Jailer and Goldbaum (59) modified the method for thiopental by employing chloroform for the extraction and by making the measurement of the ultraviolet absorption in chloroform or preferably in sodium hydroxide solution. Subsequently Walker (127) and Goldbaum (44) described fairly similar procedures for the determination of 5,5-disubstituted barbituric acids. More recently Gould and coworkers (45) have reported the use of continuous extraction with ether instead of multiple extraction with chloroform, but further details of their procedure are lacking.

The factor limiting the sensitivity of the ultraviolet spectrophotometric procedures is the ratio of the amount of barbiturates to the amount of other tissue "chromogens" concomitantly extracted. With the methods of Walker and of Goldbaum the minimal concentration of barbiturates in blood to yield satisfactory results is about 0.4 mgm per 100 ml. Samples of blood from 0.5 to 5 ml depending on the concentration of barbiturates are sufficient. The method of Goldbaum is sensitive to 1.0 mgm of barbiturate per 100 gm of tissue. Samples of tissue of 0.5 to 5 gm are employed. In the absence of other drugs with absorp-

to each other as well as to the barbiturate present, if quantitative results are to be obtained. On the basis of this observation he felt that the reaction should be used only as a qualitative test or for semiquantitative purposes.

Other investigators do not appear to be in complete agreement with these observations. Using a photoelectric colorimeter, Krause (88) found that the method of Koppanyi gave an average variation in color intensity of 7 per cent in duplicate determinations on standard Amytal solutions in the concentration range of 18 to 300 mgm per cent. However, by a suitable choice of amounts and concentrations of the reagents, he was able to reduce the average error of duplicate determinations to 1.5 per cent for the same range of concentrations. Krause believed that the results obtained by Kozelka were due largely to the use of sub-optimal amounts of reagents. Cohen (21) has also studied the optimal conditions for the cobalt color. He found that the stability of the color was satisfactory and that by observing certain precautions the method could be used for the quantitative determination of barbiturates.

Koppanyi's procedure for the determination of barbiturates in urine consisted of treatment of the urine with sodium hydroxide and copper sulfate, removal of the copper hydroxide precipitate by filtration and subsequent extraction of the filtrate with ten volumes of chloroform. Tissues containing barbiturate were liquefied with sodium hydroxide, then treated with copper sulfate and filtered, the filtrate was then acidified and extracted with chloroform. Some criticism has been directed toward these methods. Iversen (58) contended that purification through the formation of a voluminous copper hydroxide precipitate in urine involves a loss of barbiturate. Kozelka (84) has reported that the Koppanyi procedure for tissues gives low recovery of added barbiturates. Moreover, the above authors and others have objected to the large amount of chloroform which is needed. Ryley (112) and Krause (88) have shown that chloroform extracts of urine that contain no barbiturates give a certain amount of color due to urinary pigments and chromogenic substances. However, the copper hydroxide purification was not employed by these workers. Koppanyi maintains that the copper hydroxide procedure, when properly executed, gives satisfactory results, but he has also described alternative methods (46, 90). A few workers (8, 97, 108) have tried to use chromatographic adsorption in connection with the cobalt color method, but their studies have not been exhaustive enough to contribute much to an improved quantitative method. Cohen (21) has recently described a rather laborious method for the extraction of barbiturates on the basis of experiments in which barbiturates were added to tissues and body fluids and subsequently analyzed by the cobalt color method. He reported a recovery of 93 per cent or more, but the amount of added barbiturate was large (5 to 40 mgm) and he does not mention the quantity of tissues or fluids employed.

It would appear from the information available at present that with proper technic the cobalt color method is suitable for the study of the concentration of *barbital* in body tissues and fluids under the conditions of usual dosage. On the other hand, the method has been shown to yield erroneous results when used to study barbiturates which are degraded *in vivo*. For example, using the colorimetric

salts of thiopental, Thioethamyl and Evipal, whereas the corresponding ratio of rectal to intravenous doses was 2.2 to 3.1. The oral intravenous LD<sub>50</sub> ratio of a longer-acting barbiturate, sodium pentobarbital, was 6.1, and the rectal intravenous LD<sub>50</sub> was 1.4. It would be expected that sodium barbital, which undergoes virtually no destruction in the liver or other tissues, would be found to differ little in potency by all three routes.

Barbital, unlike all barbiturates with shorter duration of action, has a slow onset of action (15 minutes or more) following the intravenous injection of an anesthetizing dose. Bush (9) estimated the ratio of free acid to sodium salt of several representative barbiturates. This ratio for both barbital and Amytal was 2.5:1 at pH 7.5. Since with intravenous administration there is a long latent period for barbital and a short one for Amytal, Bush's determinations gave no support to the view of Klimesch (67) and Starkenstein and Klimesch (119) that after barbiturates which cause immediate narcosis, the lipid-soluble free acid is relatively more abundant in the plasma. However, it does appear likely that delayed equilibrium between plasma and nervous tissue accounts for the long latent period of action of barbital (*cf.*, the serial blood determinations of Dille *et al.* (28)).

The briefly summarized *in vitro* experiments of Bennhold with Evipal (6) led him to the conclusion that both plasma albumins and globulins bind the drug when it is transported intravascularly.

Except for barbital, which probably undergoes no degradation in the body, the relationship of estimated blood level to pharmacological effect has to be accepted with caution since most methods do not convincingly distinguish between the administered barbiturate and metabolically altered derivatives which, at present, appear also to be barbiturates. (An exception is the gravimetric method used by Tatum, Nelson and Kozelka [122].) Even if levels in the blood are accurately determined, their correlation with pharmacological response is best for short and very short acting drugs and poorest for a long acting barbiturate such as barbital. Dille, Linegar and Koppányi (28) reported that, after the intravenous injection of 225 mgm per kgm of sodium barbital into a dog, the blood level of the drug fell from 39 to 25 mgm per cent during the first 10 minutes at which time it can be assumed that the dog was not yet anesthetized. It is probable that the dog was anesthetized during the succeeding 1 to 5 hours when the blood level was 12 to 15 mgm per cent (similar to a dog receiving 300 mgm per kgm). Also, after recovery of consciousness in a human with barbital poisoning, the blood level may be only slightly below that of a similarly poisoned patient in coma (7.6 compared with 8.4 mgm per cent [35]), a fact which again suggests that the blood level of barbital does not always accurately reflect the level in the central nervous system. On the other hand, using Amytal, a short-acting barbiturate, Tatum, Nelson and Kozelka (122) observed that rabbits regain their righting reflexes at about the same blood level (2.9 mgm per cent), although there were wide differences in the duration of anesthesia. Data from the single case of Jailer and Goldbaum (59) suggests that it may be possible to relate

tion in the ultraviolet, these procedures are perhaps 4 times as sensitive as the cobalt color method (127)

The ultraviolet absorption methods measure the total amount of barbiturates in the extracts, and hence are subject to the same criticism as the cobalt color methods. Their use can give much valuable information about the metabolism of the barbiturates, but until the intermediary metabolism of these drugs is more thoroughly understood one cannot be certain to what extent metabolites containing the barbituric acid ring are being measured as unchanged drug.

#### (4) *Isotopic Methods*

Only two short papers have appeared on the use of isotopes for the study of the metabolism of the barbiturates (112a, 125). Isotopic methods have the advantage that they can yield information about the extent to which at least a portion of the drug is degraded to compounds which enter the metabolic pool. For example, van Dyke and coworkers (125) examined the excess  $N^{15}$  in the urinary ammonia and urea after the oral administration of pentobarbital labeled with  $N^{15}$  and found that less than 8 per cent of the isotope was contained in these fractions. Furthermore, the amount of unchanged drug and of the metabolites can be determined with precision by the method of isotope dilution. The disadvantages of the isotope methods are the expense of the isotopes, the organic synthesis required and the special equipment necessary for the quantitative determination of the isotopes.

#### (5) *Pharmacological Methods*

Several investigators have tested extracts of urine for hypnotic activity in mice or rats after the administration of barbiturates. This method has been used principally for the study of barbiturates which are largely degraded *in vivo* and yield only very small amounts of unchanged drug in the urine (55, 118), or to confirm or disprove data obtained with the cobalt color method (8, 74). The results of pharmacological testing are at best very crude. Furthermore, there is always the possibility that certain of the metabolites of the barbiturates possess some hypnotic activity.

Other pharmacological methods have been used in elucidating specific features of barbiturate metabolism which are discussed below.

#### ABSORPTION AND DISTRIBUTION IN BODY FLUIDS

The absorption of barbiturates occurs readily from the gastro-intestinal tract. According to Weese (128), a considerable part of a dose can be absorbed from the stomach, and narcosis can follow intragastric administration to guinea pigs with a ligature around the pylorus. Most authors agree that rectal administration is much more efficient than the oral route, thus avoiding immediate passage through the liver which is the most important organ in degrading all but a few of the barbiturates. For example, Werner, Pratt and Tatum (130) using rabbits found that the ratio of the oral to the intravenous  $LD_{50}$  was 15:1 or more for the sodium

salts of thiopental, Thioethamyl and Evipal, whereas the corresponding ratio of rectal to intravenous doses was 2.2 to 3.1. The oral intravenous  $LD_{50}$  ratio of a longer-acting barbiturate, sodium pentobarbital, was 6.1, and the rectal intravenous  $LD_{50}$  was 1.4. It would be expected that sodium barbital, which undergoes virtually no destruction in the liver or other tissues, would be found to differ little in potency by all three routes.

Barbital, unlike all barbiturates with shorter duration of action, has a slow onset of action (15 minutes or more) following the intravenous injection of an anesthetizing dose. Bush (9) estimated the ratio of free acid to sodium salt of several representative barbiturates. This ratio for both barbital and Amytal was 2.5:1 at pH 7.5. Since with intravenous administration there is a long latent period for barbital and a short one for Amytal, Bush's determinations gave no support to the view of Klimesch (67) and Starkenstein and Klimesch (119) that after barbiturates which cause immediate narcosis, the lipid-soluble free acid is relatively more abundant in the plasma. However, it does appear likely that delayed equilibrium between plasma and nervous tissue accounts for the long latent period of action of barbital (*cf.*, the serial blood determinations of Dille *et al.* (28)).

The briefly summarized *in vitro* experiments of Bennhold with Evipal (6) led him to the conclusion that both plasma albumins and globulins bind the drug when it is transported intravascularly.

Except for barbital, which probably undergoes no degradation in the body, the relationship of estimated blood level to pharmacological effect has to be accepted with caution since most methods do not convincingly distinguish between the administered barbiturate and metabolically altered derivatives which, at present, appear also to be barbiturates. (An exception is the gravimetric method used by Tatum, Nelson and Kozelka [122].) Even if levels in the blood are accurately determined, their correlation with pharmacological response is best for short and very short acting drugs and poorest for a long acting barbiturate such as barbital. Dille, Linegar and Koppanyi (28) reported that, after the intravenous injection of 225 mgm per kgm of sodium barbital into a dog, the blood level of the drug fell from 39 to 25 mgm per cent during the first 10 minutes at which time it can be assumed that the dog was not yet anesthetized. It is probable that the dog was anesthetized during the succeeding 1 to 5 hours when the blood level was 12 to 15 mgm per cent (similar to a dog receiving 300 mgm per kgm). Also, after recovery of consciousness in a human with barbital poisoning, the blood level may be only slightly below that of a similarly poisoned patient in coma (7.6 compared with 8.4 mgm per cent [35]), a fact which again suggests that the blood level of barbital does not always accurately reflect the level in the central nervous system. On the other hand, using Amytal, a short-acting barbiturate, Tatum, Nelson and Kozelka (122) observed that rabbits regain their righting reflexes at about the same blood level (2.9 mgm per cent), although there were wide differences in the duration of anesthesia. Data from the single case of Jailer and Goldbaum (59) suggests that it may be possible to relate

tion in the ultraviolet, these procedures are perhaps 4 times as sensitive as the cobalt color method (127)

The ultraviolet absorption methods measure the total amount of barbiturates in the extracts, and hence are subject to the same criticism as the cobalt color methods. Their use can give much valuable information about the metabolism of the barbiturates, but until the intermediary metabolism of these drugs is more thoroughly understood one cannot be certain to what extent metabolites containing the barbituric acid ring are being measured as unchanged drug.

#### (4) *Isotopic Methods*

Only two short papers have appeared on the use of isotopes for the study of the metabolism of the barbiturates (112a, 125). Isotopic methods have the advantage that they can yield information about the extent to which at least a portion of the drug is degraded to compounds which enter the metabolic pool. For example, van Dyke and coworkers (125) examined the excess  $N^{15}$  in the urinary ammonia and urea after the oral administration of pentobarbital labeled with  $N^{15}$  and found that less than 8 per cent of the isotope was contained in these fractions. Furthermore, the amount of unchanged drug and of the metabolites can be determined with precision by the method of isotope dilution. The disadvantages of the isotope methods are the expense of the isotopes, the organic syntheses required and the special equipment necessary for the quantitative determination of the isotopes.

#### (5) *Pharmacological Methods*

Several investigators have tested extracts of urine for hypnotic activity in mice or rats after the administration of barbiturates. This method has been used principally for the study of barbiturates which are largely degraded *in vivo* and yield only very small amounts of unchanged drug in the urine (55, 118), or to confirm or disprove data obtained with the cobalt color method (8, 74). The results of pharmacological testing are at best very crude. Furthermore, there is always the possibility that certain of the metabolites of the barbiturates possess some hypnotic activity.

Other pharmacological methods have been used in elucidating specific features of barbiturate metabolism which are discussed below.

#### ABSORPTION AND DISTRIBUTION IN BODY FLUIDS

The absorption of barbiturates occurs readily from the gastro-intestinal tract. According to Weese (128), a considerable part of a dose can be absorbed from the stomach, and narcosis can follow intragastric administration to guinea pigs with a ligature around the pylorus. Most authors agree that rectal administration is much more efficient than the oral route, this avoids immediate passage through the liver which is the most important organ in degrading all but a few of the barbiturates. For example, Werner, Pratt and Tatum (130) using rabbits found that the ratio of the oral to the intravenous  $LD_{50}$  was 15:1 or more for the sodium

gravimetric method used by Kozelka and Tatum (86) revealed 0.20 mgm per cent of phenobarbital in human cerebrospinal fluid 30 to 45 minutes after the oral administration of 585 mgm of the drug. Even larger doses of various barbiturates, particularly barbital (up to 10 gm) and phenobarbital (up to 6 to 7 gm) had been ingested by the patients or subjects of Purves-Stewart and Willcox (107) and Fretwurst and Voss (42). Samples of cerebrospinal fluid were investigated 3 to 35 hours after the drug was taken. For example, Purves-Stewart and Willcox, using an undisclosed method, observed 7.7 to 9.0 mgm. per cent of phenobarbital in the cisternal fluid with little change from the sixteenth to the thirty-fifth hour. Fretwurst and Voss found little difference in the concentration of barbital or phenobarbital in the plasma as compared with the cerebrospinal fluid. One patient is reported to have taken 800 mgm of phenobarbital (compared with 585 mgm for the patients of Kozelka and Tatum). Nineteen hours later the plasma and cerebrospinal fluid contained, respectively, 4.2 and 3.1 mgm per cent of the drug. If it be assumed that the patient had an extracellular fluid volume of about 12 l, this fluid would have contained about 60 per cent of the dose despite excretion, destruction and tissue localization or binding. This seems very unlikely. Vogt injected barbital or Dial into dogs and consistently found less drug in the cerebrospinal fluid than in various parts of the central nervous system (126).

#### DISTRIBUTION IN TISSUES

After absorption or intravenous injection, barbiturates probably enter all cells and are actually concentrated or localized in some tissues such as the liver and kidneys. This distribution throughout all tissues is illustrated by the appearance of barbital in the gastric juice and pancreatic secretion (20) and in the fetal tissues (25, 26). Phenobarbital can be detected in human milk after a single dose of 100 mgm (124). It is likely that equilibrium, however unstable, is achieved more quickly for short-acting barbiturates than for long-acting drugs, especially barbital with its long latent period of action. Only barbital undergoes no metabolic change, and since the principal degradation products of the other drugs are probably pharmacologically inactive barbiturates, colorimetric or ultraviolet spectrophotometric determinations of drugs other than barbital in tissues yield little reliable information concerning the actual concentration of injected drug, especially after intervals of an hour or more. This objection does not hold for the gravimetric method of Kozelka and his colleagues who actually identified the barbiturate after isolation (84, 86, 87). Apparently little degradation of phenobarbital, Amytal, Neonal, or pentobarbital occurs within 15 minutes after intraperitoneal injection of large doses into rats, since practically all the injected barbiturate can be recovered and identified (84). On the other hand, within three hours after the oral administration of pentobarbital to dogs, the urine contains considerable amounts of degraded pentobarbital, probably in the form of barbiturate, and almost no unaltered drug (96, 125).

The concentration of barbital in tissues after the administration of doses ranging from innocuous to fatal has been investigated. Among the first careful toxic-

depth of anesthesia with the blood level of thiopental. The apparent relationship between the blood levels of barbiturates and pharmacological effect is listed in Table 1.

Anderson and Essex (1) confirmed the work reported in the unpublished thesis of Delmonico who found cyclic fluctuations in the blood level of barbiturate in dogs. Anderson and Essex injected either Amytal or pentobarbital and determined barbiturate colorimetrically by Levvy's method (89) after having concluded that Delmonico's method was unreliable. Even more unusual was their finding that cyclic disappearance and reappearance of pentobarbital occurs in the

TABLE 1

*Apparent Relationship between Blood Levels of Barbiturates and Pharmacological Effect*

BARBITURATE	METHOD*	ANIMAL	PHARMACOLOGICAL EFFECT		REFERENCE
			Coma or surgical anesthesia	Respiratory arrest	
			mgm per cent	mgm per cent	
Barbital	C	Dog	12-15†		28
Barbital	U	Dog	15-18		35
Barbital	U	Man†	9-21		35
Phenobarbital	U	Rabbit	7-17		44
Phenobarbital	U	Man	5-10		44
Phenobarbital	U	Man	10-13		35
Amytal	U	Rabbit	7		44
Amytal	G	Rabbit	3		122
Pentobarbital	U	Rabbit	4-5-7		44
Seconal	U	Rabbit	6-5		44
Thiopental	C	Rabbit	4-8	7-4	19
Thiopental	U	Man	2-0-3-0		59
Kemithal	C	Rabbit	8-8	19	19
Kemithal	C	Man	2-3-4-5		19

\* C = colorimetric, G = gravimetric, and U = ultraviolet spectrophotometric

† One to 5 hours after anesthetic dose

‡ After a therapeutic dose of barbital, the blood level was 5.0-5.5 mgm per cent by a colorimetric method (89)

heart-lung preparation with or without kidneys or extremities or both in the circulation. No other investigator has observed "cyclic disappearance from and reappearance in the blood" of intravenously injected barbiturate.

According to Goldbaum (44) the level of various barbiturates is slightly if at all higher in the plasma than in the whole blood of rabbits. Yet Fretwurst and Voss (42), investigating human blood in cases of poisoning by barbital and phenobarbital (drugs used by Goldbaum), asserted that erythrocytes contain virtually no barbiturate.

There are serious discrepancies in the reports on the concentration of barbiturates in cerebrospinal fluid. The differences appear in part to depend upon variations in dose and in interval between administration and sampling. The



gravimetric method used by Kozelka and Tatum (86) revealed 0.20 mgm per cent of phenobarbital in human cerebrospinal fluid 30 to 45 minutes after the oral administration of 585 mgm of the drug. Even larger doses of various barbiturates, particularly barbital (up to 10 gm) and phenobarbital (up to 6 to 7 gm) had been ingested by the patients or subjects of Purves-Stewart and Willcox (107) and Fretwurst and Voss (42). Samples of cerebrospinal fluid were investigated 3 to 35 hours after the drug was taken. For example, Purves-Stewart and Willcox, using an undisclosed method, observed 7.7 to 9.0 mgm per cent of phenobarbital in the cisternal fluid with little change from the sixteenth to the thirty-fifth hour. Fretwurst and Voss found little difference in the concentration of barbital or phenobarbital in the plasma as compared with the cerebrospinal fluid. One patient is reported to have taken 800 mgm of phenobarbital (compared with 585 mgm for the patients of Kozelka and Tatum). Nineteen hours later the plasma and cerebrospinal fluid contained, respectively, 4.2 and 3.1 mgm per cent of the drug. If it be assumed that the patient had an extracellular fluid volume of about 12 l, this fluid would have contained about 60 per cent of the dose despite excretion, destruction and tissue localization or binding. This seems very unlikely. Vogt injected barbital or Dial into dogs and consistently found less drug in the cerebrospinal fluid than in various parts of the central nervous system (126).

#### DISTRIBUTION IN TISSUES

After absorption or intravenous injection, barbiturates probably enter all cells and are actually concentrated or localized in some tissues such as the liver and kidneys. This distribution throughout all tissues is illustrated by the appearance of barbital in the gastric juice and pancreatic secretion (20) and in the fetal tissues (25, 26). Phenobarbital can be detected in human milk after a single dose of 100 mgm (124). It is likely that equilibrium, however unstable, is achieved more quickly for short-acting barbiturates than for long-acting drugs, especially barbital with its long latent period of action. Only barbital undergoes no metabolic change, and since the principal degradation products of the other drugs are probably pharmacologically inactive barbiturates, colorimetric or ultraviolet spectrophotometric determinations of drugs other than barbital in tissues yield little reliable information concerning the actual concentration of injected drug, especially after intervals of an hour or more. This objection does not hold for the gravimetric method of Kozelka and his colleagues who actually identified the barbiturate after isolation (84, 86, 87). Apparently little degradation of phenobarbital, Amytal, Neonol, or pentobarbital occurs within 15 minutes after intraperitoneal injection of large doses into rats, since practically all the injected barbiturate can be recovered and identified (84). On the other hand, within three hours after the oral administration of pentobarbital to dogs, the urine contains considerable amounts of degraded pentobarbital, probably in the form of barbiturate, and almost no unaltered drug (96, 125).

The concentration of barbital in tissues after the administration of doses ranging from innocuous to fatal has been investigated. Among the first careful toxico-

logical reports in man is that of Pucher (105) who found the following concentrations of barbital in the tissues after fatal poisoning from a dose of 6 gm kidneys 19.4, brain 14.0, liver 12.8 and spleen 12.1 (all expressed as mgm per 100 gm). Similar observations were made by Zwikker and Steenhauer (133). Experimental studies have largely been performed in mammals. Dille, Linegar and Koppanyi (28) concluded that the uptake of barbital by the tissues is relatively faster at low than at high blood concentrations, a finding which is not surprising. Tissue concentrations in comparison with blood concentrations varied with the dose and with the interval between injection and sampling. An example is the authors' dog number 3, from which samples were taken 22 hours after the injection of 300 mgm per kgm of sodium barbital: blood 8.0, various divisions of brain 2.0 to 7.0, liver 14.0 and kidney 17.5 (all expressed as mgm per 100 cc or 100 gm). Other examples of tissue analyses will be found in several reports (2, 71, 72, 77, 79, 126).

Experiments with barbital alone should be considered in any attempt to decide whether there is significant localization of barbiturate either in the central nervous system or specifically in the diencephalon where "centers" regulating the degree of wakefulness or sleepiness are believed to reside. During the period 1927-1937, E and I Keeser contended that after small doses of barbital (e.g., 25 mgm per kgm), intravenously administered to rabbits or dogs, specific localization of the drug occurred in the diencephalon, as revealed by the presence of easily sublimed drug in crystalline form (60-65). Similar sublimation of extracts of other cerebral structures (cortex, pons and medulla, cerebellum) revealed no barbital or only a much smaller quantity (mesencephalon). No quantitative data were offered. M. Vogt (126) made a careful attempt to secure quantitative evidence of such specific localization. Her methods permitted recovery of about 50 per cent of large doses of drug, but were only qualitative with doses of 10 to 100 mgm per kgm. She found no evidence of localization of barbital in a specific division of the brain.

Work which might be cited as favoring localization in the brain in comparison with other tissues (e.g., 31 and 32) contains no adequate description of methods. In fact, Fabre in 1925 reported relatively enormous *total amounts* of barbital in the brain in comparison with other tissues such as the liver (31, 32), yet in 1934 in another study (30) he stated that the *concentration* of barbital in the liver was higher than in the brain. All other work supports the contention of Vogt and of Koppanyi and his colleagues that barbital is not specifically localized in the brain and, more often than not, is there in lower concentration than in such tissues as the liver, kidney and spleen (2, 71, 72, 77, 105, 128, 129, 133).

The cross-circulation experiments of Koppanyi and Linegar (76) indicated that as the blood level of barbital fell, the drug diffused from the central nervous system and rapid recovery could follow. It would seem that barbital is loosely bound to nervous tissue and probably to other tissues as well.

There are reports of tissue distribution of other barbiturates by methods, either colorimetric or ultraviolet spectrophotometric, which probably cannot distinguish the drugs from closely related degradation products. The drugs studied were phenobarbital (44, 72, 77), Amytal (25, 44), Dial (72, 126), Sandoptal (72),

Phanodorn (126), pentobarbital (28, 44, 72, 77), Seconal (44) and thiopental (59) A careful gravimetric method has also been used for Amytal (87, 122)

#### DETOXIFICATION

The detoxification of barbiturates depends upon renal excretion or destruction in the tissues or both Barbitol is the only barbiturate which appears to leave the body without alteration, and its detoxification depends, in a practical sense, entirely on renal excretion, this may extend over days (4, 33, 95) Excretion of barbitol may be markedly reduced by renal disease (3) and may be so impaired in animals with experimental nephritis (potassium chromate, tartaric acid or uranium acetate) that anesthetic doses lead to death (100) The period of survival after total nephrectomy is greatly shortened if barbitol has been administered (56, 94) Although phenobarbital is in a large part degraded in the body in some unknown fashion, experimental studies indicate that functioning kidneys are necessary for its detoxification (56, 94) It has not been demonstrated that the kidneys play an *essential* part in the detoxification of the other barbiturates

The most important other single organ for detoxification, as would be expected, is the liver Liver damage by chloroform, carbon tetrachloride or phosphorus markedly increases the toxicity of barbiturates such as Amytal, pentobarbital and Evipal (18, 66, 93, 103, 104, 122), whereas this increase is not observed after nephrectomy (56, 128) In the experiments of Tatum, Nelson and Kozelka (122), in which the liver was damaged with carbon tetrachloride, unusual amounts of Amytal persisted in the liver until delayed detoxification finally permitted recovery Partial hepatectomy, with proper precautions, has the same effect on all the common short-acting barbiturates used orally (94, 114)

The extent to which other tissues participate in the degradation of barbiturates appears to be less important Assaying Evipal biologically, Martin, Herrlich and Clark (93) observed that destruction of the drug, especially by liver and less strikingly by skeletal muscle and spleen, occurred *in vitro*; but that no such destruction by kidney, brain and oxygenated blood could be demonstrated Likewise from *in vitro* experiments, Dorfman and Goldbaum (29) concluded that kidney, skeletal muscle and brain do not destroy a number of barbiturates which are degraded to a varying extent by liver However, they relied upon an ultraviolet spectrophotometric method which perhaps yields more significant results concerning disappearance than persistence of drug Masson and Beland (94) believed that the kidney participates as much as the liver in the detoxification of Dial, Neonol, Phanodorn and Delvinal, however, Hirschfelder and Haury (56) who also performed experiments with the first three drugs of this group observed no change in potency after nephrectomy

Apparently the liver inactivates a thiobarbiturate like thiopental so efficiently that extensive reduction of functional hepatic tissue is necessary before the liver's importance in this regard can be revealed Masson and Beland (94) denied that hepatic degradation of thiopental occurs, however, accumulating data reveal that this is an erroneous interpretation and suggest that a sufficiently large

proportion of the liver had not been removed from their rats Shideman, Kelly and Adams (117) made Eck fistulas in rats and damaged the livers of mice with carbon tetrachloride They had no difficulty in demonstrating a marked increase in the potency of thiobarbiturates (thiopental, Surital and Thioethamyl) and of pentobarbital in such animals The potency of barbital was not changed Hepatic tissue *in vitro* will likewise degrade thiobarbiturates (29, 117) Lastly, patients with extensive hepatic disease may be anesthetized for remarkably long periods by thiopental (99, 117) Experiments in dogs with Eck fistulas and experiments with the heart-lung preparation with or without liver or kidney continue to support the view that the principal degradation of thiopental occurs in the liver Some destruction occurred in the heart-lung preparation and more destruction was found when the kidney was in the circulatory circuit, in agreement with findings *in vitro* (29, 66)

A sex difference in response to some barbiturates—adult females are more susceptible than adult males—has been reported by Holck as well as by previous investigators (57) Susceptibility to pentobarbital, measured by sleeping time, is increased after spaying or castration The injection of testosterone into castrated male rats can reduce susceptibility to pentobarbital (16, 17, 98). Those interested in the rate of detoxification of barbiturate in intact mammals are referred to reports by Koppányi and Liberson (75), Kohn and Grimes (69) and Das and Raventós (23) The drugs used were Amytal, barbital, Evipal, Kemithal, pentobarbital and thiopental Das and Raventós (23) concluded that a very short-acting barbiturate like Evipal is detoxified in an exponential manner, i.e. a constant proportion ( $1/35$  to  $1/30$ ) of the amount of the drug present in the body is inactivated per minute.

#### EXCRETION AND METABOLIC FATE

It is known that the body possesses two methods for removing the barbiturates (1) destruction or chemical alteration principally in the liver, and (2) excretion through the kidneys One barbiturate at least is largely excreted as such, others are destroyed in the body, and still others are partly excreted and partly destroyed The fate of a particular barbiturate depends upon its chemical constitution, but at present one cannot predict from chemical structure precisely how the drug will be metabolized

The metabolic fate of the barbiturates will be discussed with reference to a familiar classification based on the duration of hypnotic or anesthetic action (Table 2) This is a desirable approach, since there is some correlation between the *in vivo* stability of the individual barbiturates and their duration of action As a matter of fact, the duration of action may be used as a biological test of stability On this basis, the long-acting drugs, barbital, phenobarbital and Dial, appear to be relatively stable, and, indeed, they do appear in the urine in detectable and measurable quantities On the other hand, those members of the series which, on the basis of a relatively short period of action, are more unstable appear in the urine only in very small amounts except under unusual conditions

The barbiturate which has received most study is barbital Fischer and von

TABLE 2  
*Metabolic Fate of Barbiturates*

BARBITURATE	% UN-CHANGED BARBITURATE EXCRETED	METHOD FOR DETECTION*	METABOLITE	% METABOLITE EXCRETED
<i>Drugs with Long Duration of Effect</i>				
Alurate	13-24	G (38, 68, 102, 111)	?	
Barbital	65-90	G (4, 33, 34, 47, 95, 111, 115) C (79)		
Dial	27-31	G (101, 110)	?	
Phenobarbital	11-25	G (47, 101, 111) C (79)	?	
Rutonal	25	G (101)	?	
<i>Drugs with Moderate Duration of Effect</i>				
Amytal	0	G (53, 55, 118) P (8, 55, 118)	?	
Butisol		Not adequately studied		
Delvinal		Not adequately studied		
Dormovit	2-3	G (41)	?	
Ipral		Not adequately studied		
Medomin	0	G (106)	5-cycloheptenonyl, 5-ethyl barbituric acid	1-5
Neonal	0	G (53, 55)	?	
Nostal	1-3	G (47) B (131)	5-acetonyl, 5-isopropylbarbituric acid (47)	6-16
Ortal	0	P (8)		
Pentobarbital	0-3	G (54, 55, 118) P (8, 55, 118) I (125, 112A)	5-ethyl, 5(3-hydroxy-1-methylbutyl) barbituric acid (96)	?
Pernoston	0 1-0 5	G (39)	5-acetonyl, 5-sec-butylbarbituric acid	5-17
Phanodorn	2-7	G (40)	5-cyclohexenonyl, 5-ethylbarbituric acid	12-19
Sandoptal	0	C (74)	?	
<i>Drugs with Short Duration of Effect</i>				
Eunarcon	0	C (43) B (43)	?	
Narconumal	0	G (10)	5-allyl, 5-isopropyl barbituric acid	Trace
Prominal	0	G (15)	5'-ethyl, 5-phenylbarbituric acid	4
Seconal		Not adequately studied		

TABLE 2—Continued

BARBITURATE	% OR CHANGED BARBITURATE EXCRETED	METHOD FOR DETECTION*	METABOLITE	% META- LITE EXCRET
Drugs with "Ultrashort" Duration of Effect				
Evipal	0	P (12)	5-cyclohexenonyl, 5-methylbarbituric acid	5
			isomeric 5-cyclohexenonyl, 1, 5-dimethylbarbituric acids (13)	?
Kemithal Thioethamyl	0.8-2	C (19) Not adequately studied	?	
Thiopental	Traces	U (59)	?	

\* G = Gravimetric method (chemical isolation)

C = Colorimetric method

I = Isotopic method

U = Ultraviolet spectrophotometric method

P = Pharmacological method (bio-assay)

B = Determination of organic bromine

Figures in parentheses are references

Mering (33) administered 4 gm in 48 hours to a young man and found that about 70 per cent of the drug was excreted over a period of five days. Later studies (34, 47, 79, 111, 115) in which the gravimetric or cobalt color methods were used have shown that 65 to 90 per cent of a dose of barbital administered to man, dog, cat or rabbit is excreted in the urine. Only very small amounts are excreted in the feces. Normal humans eliminate about 8 per cent of the drug in the urine in the first 12 hours, 20 per cent in 24 hours, and 35 per cent in 48 hours (109). It has been demonstrated that traces of barbital are still detectable in the urine 8 to 12 days after the administration of a hypnotic dose (95). Bachem (4) reported that 90 per cent of small doses but only about 45 to 50 per cent of large doses is excreted. This work has been refuted by Mattisson (95) and Koppanyi (79).

Koppanyi and coworkers (78) studied the action of barbital in fowls and turtles. Fowls receiving anesthetic doses of the drug did not recover from the narcosis and eventually died of respiratory failure. The fowl could recover from anesthetic doses of those barbiturates which in the mammal depend only in part (phenobarbital) or not at all on renal excretion (pentobarbital, Neonal and Pernoston). With smaller doses, the amount of barbital excreted by the fowl was less than half of that excreted by the mammal. Furthermore, the period of excretion was greatly prolonged in the fowl. Turtles usually recovered from anesthetic doses of barbital, but did so very slowly. They excreted only a very small amount of barbital (10 to 25 per cent of the doses) over a period of weeks and appeared to show retention of the drug in the blood and organs 3 to 11 weeks after the administration of a single dose.

The other long-acting barbiturates appear to suffer some change in the animal body. The elimination of phenobarbital in the urine of man and dogs has been studied by different workers (47, 79, 101, 111) with good agreement in results. Dogs given single doses of 68 to 100 mgm per kgm and humans given 250 mgm to 1.8 gm daily over a period of several days excreted 11 to 25 per cent of the dose in the urine. The excretion may continue for as long as ten days after the administration of the drug (36). The closely related barbiturate, Rutonal (5-methyl, 5-phenylbarbituric acid) appears to be eliminated in a very similar way. Paget and Desodt (101) reported that the urine of a patient given 200 mgm per day for 14 days contained 25 per cent of the drug.

The excretion of Dial has been studied less extensively than that of barbital and phenobarbital. Reiche and Halberkann (110) found that 3 patients given 100 to 300 mgm daily for 4 to 11 days excreted 27 to 31 per cent of the drug in the urine. The Dial was excreted for 7 days after administration was stopped. Paget and Desodt (101) studied the urine of a patient given 100 mgm per day for 13 days and found 30 per cent of the administered drug. Koppányi and coworkers (79) gave a dog a single dose of 80 mgm per kgm intravenously and with the cobalt color method found that 40 per cent of the drug was excreted in 2.5 days.

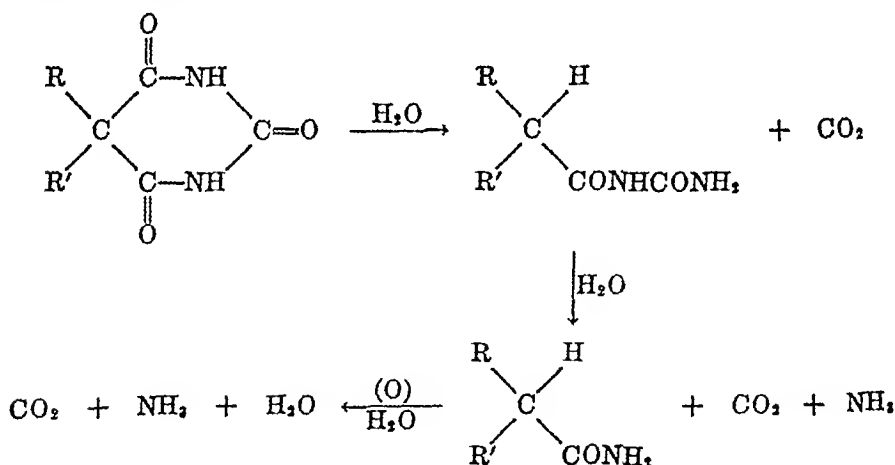
The elimination of Alurate in the urine has been studied after single doses in dogs and after repeated doses in humans. The results of most workers (38, 68, 102, 111) indicate an excretion of 13 to 24 per cent of the drug. With continued administration the unchanged barbiturate may be found in the urine 3 to 5 days after the drug is discontinued. In febrile patients the excretion of Alurate appears to be only 4 to 6 per cent (38). Fabre and Fredet (31, 32) have reported results which are in marked contrast with those of other investigators. They asserted that 46 to 90 per cent of doses of 0.55 to 1.0 gm was eliminated by humans in 6 days. Furthermore, they stated that their product was identified by its melting point and by preparation of the dioxanthyl derivative.

It will be noted that none of the barbiturates with persistent action contains an alkyl or alkenyl side-chain with more than three carbon atoms. It seems to be true that barbiturates with two short alkyl or alkenyl chains are relatively stable. Increasing the length of one of the chains increases the activity, but the molecule is then more susceptible to chemical change in the liver. In this way drugs of short or moderate duration of action are obtained. The addition of a methylene group to Alurate yields Sandoptal, a drug with moderate duration of effect. In contrast with Alurate, which is excreted to the extent of 20 per cent, Sandoptal is eliminated in the urine in negligible amounts (74). Likewise, the addition of two methylene groups to phenobarbital yields 5-*n*-butyl, 5-phenylbarbituric acid, whereas phenobarbital is excreted to the extent of 25 per cent, *n*-butylphenylbarbituric acid cannot be detected in urine (74). Nothing is known concerning the fate of Sandoptal or *n*-butyl phenylbarbituric acid.

There is some disagreement concerning the excretion of Neonal. Koppányi and Krop (74), using the cobalt color method, concluded that dogs eliminate 5 to 41 per cent of the drug unchanged in the urine in 3 to 7 days. As was pointed out

previously, the use of the cobalt color method for the study of the excretion of drugs which are degraded *in vivo* might be expected to yield erroneous results. However, the above investigators stated that in every case the residues from the chloroform extracts of the urine had anesthetic activity in rats. Furthermore, the urine from the rats was chemically examined, and it was always possible to show the presence of barbiturates by means of the cobalt color test and to identify crystals of Neonal by microscopic examination of the dried chloroform extract. In contrast, Herwick (53, 55) was not able to isolate any unchanged Neonal from the urine of dogs given anesthetic doses, and all his extracts were inactive when injected into mice.

Amytal and Ortal appear to be eliminated in the urine in negligible amounts. The cobalt color method indicates an excretion of both of these substances in the urine (8, 74), but it is now pretty well established that the chromogenic substances are unidentified metabolites of the drugs rather than the unchanged drugs (8, 88). Herwick (53, 55) and Shonle and coworkers (118) carried out exhaustive attempts to isolate Amytal from the urine of dogs and patients given widely varying doses but obtained at most only traces of the drug. Koppanyi and Krop (74) reported that after the administration of Amytal extracts of urine possess anesthetic activity in rats. Other workers (8, 55, 118) have found no evidence for the presence of hypnotic compounds in the urine after administration of either Amytal or Ortal.

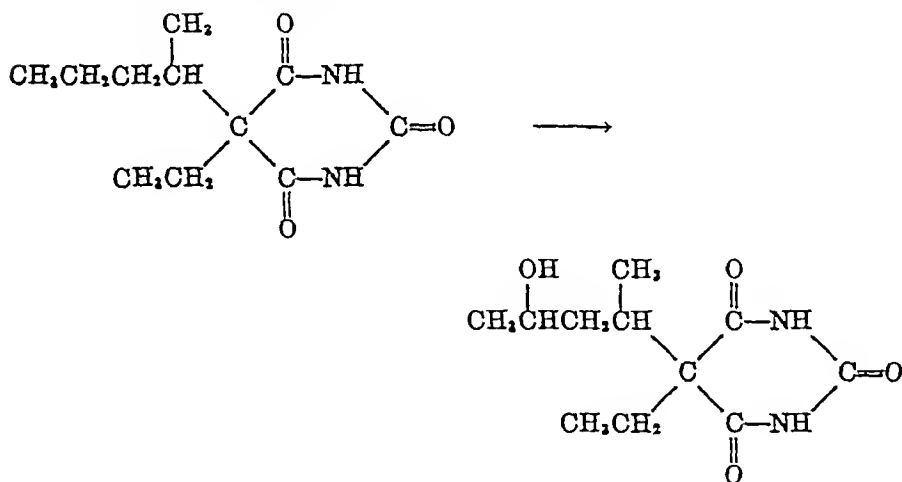


There is general agreement that only very small amounts of pentobarbital are eliminated in the urine (8, 54, 55, 118, 125). Shonle and coworkers (118) suggested that the destruction of pentobarbital and Amytal in the body involved hydrolytic cleavage of the barbituric acid ring. They explained the absence of the acetyl urea and acetamide derivatives in the urine by assuming that these compounds were completely metabolized to carbon dioxide, ammonia and water. Recently van Dyke and coworkers (125) demonstrated that such extensive degradation does not occur with pentobarbital. After the administration of pento-



barbital labeled with  $N^{15}$  they found that less than 8 per cent of the isotope was excreted as ammonia and urea

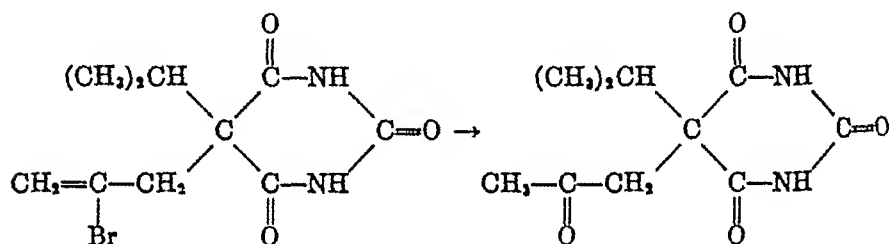
Only one metabolite of pentobarbital has been isolated in pure form and characterized Maynert and van Dyke (96) described a new barbiturate which is apparently 5-ethyl,5(3-hydroxy-1-methyl-butyl) barbituric acid Elementary analysis, ultraviolet absorption, the preparation of a crystalline acetate and the formation of iodoform with sodium hypoiodite were used to deduce the structure The compound is optically active and, hence, must be derived from only one of the enantiomorphs of pentobarbital. It has no apparent pharmacological effect in mice Herwick (54, 55) and Koppányi and coworkers (77) have detected the presence in urine of metabolites with depressant or hypnotic activity, but no pure compounds were isolated Also, Barris and Magoun (5) have reported the presence in urine of a reducing substance following injection of pentobarbital, but its identity is unknown Using filter-paper chromatography, Roth and coworkers (112a) found evidence for five radioactive metabolites in the urine of mice after the administration of pentobarbital labeled with  $C^{14}$  They stated that none of these compounds was urea or unchanged drug



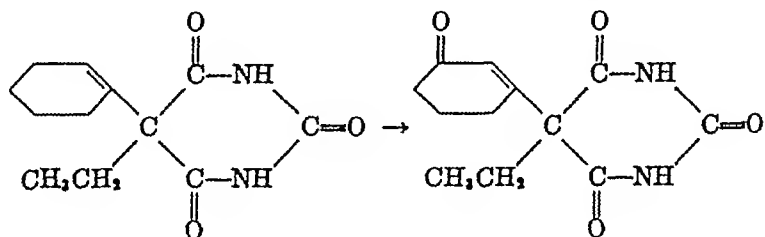
The isolation of 5-ethyl,5(3-hydroxy-1-methylbutyl) barbituric acid from urine provides the first positive clue to the metabolic fate of the dialkylbarbituric acids It would appear that ethyl groups attached to the barbituric acid ring do not suffer change in the body For example, diethylbarbituric acid is excreted unchanged Increasing the length of one of the chains forms a molecule which is more susceptible to chemical change in the liver Previously it was not known whether the change in such barbiturates was due to oxidation, hydrolysis, conjugation or a combination of these reactions It now appears likely that direct oxidative attack of side chains containing four or more carbon atoms is an important phase of the chemical alteration of such compounds in the body

Nostal and its homologue, Pernoston, provide other examples in which metabo-

lites of barbiturates have been isolated. Different investigators (47, 131) have reported that 1 to 3 per cent of single or repeated doses of Nostal is eliminated in the urine. Halberkann and Reiche (47) were able to isolate from the urine, in addition to the unchanged drug, 5-acetonyl,5-isopropylbarbituric acid in amounts which were sometimes as high as 12 to 16 per cent of the dose. Boedecker and Ludwig (7) gave the acetonyl derivative intravenously to rabbits and found that only 19 per cent of the compound was excreted unchanged. On the basis of this observation they postulated that the remainder of the compound was oxidized *in vivo* to 5-carboxymethyl,5-isopropylbarbituric acid. Although the latter compound has been prepared and shown to be without hypnotic activity, nevertheless it has never been isolated from urine. The metabolic fate of Pernoston is very similar. Although only traces of unchanged drug are excreted, 5 to 17 per cent of the dose may be accounted for as 5-acetonyl,5-*sec*-butylbarbituric acid (39).



The metabolic fate of Phanodorn was investigated by Fretwurst, Halberkann and Reiche (40). They found that 2 to 7 per cent of the drug is eliminated unchanged and that 12 to 19 per cent is excreted as a non-toxic compound which they isolated in pure form. On the basis of chemical analysis they believed that the substance was cyclohexenonyl ethylbarbituric acid, but a thorough-going investigation of its reactions and a proof of structure are still lacking.



A similar picture has evolved from work on two closely related drugs. After administration of 5-cyclohexenyl,5-methylbarbituric acid (*nor*-Evipal) to dogs, Bush and Butler (12) recovered 10 per cent of unchanged drug from the urine. Later (13) they were able to isolate a new compound in somewhat larger amounts. The elementary analysis corresponded to 5-cyclohexenonyl,5-methylbarbituric acid. The substance was relatively inactive as a hypnotic. Likewise, Pulver (106) found that 1 to 5 per cent of doses of Medomin is excreted as a non-hypnotic.

compound with the elementary composition of cycloheptenonylethylbarbituric acid. A semicarbazone derivative was prepared, and the analytical values were in accord with theory. However, in neither case has there been a complete proof of structure.

Dormovit, 5-furfuryl, 5-isopropylbarbituric acid, has had some clinical use in Europe. Fretwurst and Never (41) found that a dog and 4 patients given the drug over a period of several days eliminated 2 to 3 per cent of the total dose administered. The excretion of unchanged barbiturate was complete 2 days after administration was stopped.

The short-acting and ultra-short-acting barbiturates are N-alkyl derivatives of disubstituted barbituric acids or disubstituted derivatives of 2-thiobarbituric acid. When these compounds are given intravenously they are much more active but for a much shorter period than any of the compounds mentioned thus far. It has been found, however, that long continued intravenous injection or repeated intravenous injection of these compounds may lead to a progressive prolongation of depression, indicating that the drugs are not being rapidly and completely destroyed as might be inferred from the short period of depression or anesthesia following the initial injection. Butler and Bush (15) suggested that these substances might be transformed *in vivo* not into inactive compounds, but into less active compounds of longer duration of action. They showed that this is definitely the case with N-methylbarbital (11, 15). Following the intravenous administration of anesthetic doses of this drug into dogs, they isolated barbital from the urine in as much as 69 per cent yield, only 2 to 3 per cent of unchanged N-methyl-barbital was recovered.

In similar experiments with N-methylphenobarbital (Mebaral or Prominal), Butler and Bush isolated phenobarbital with a yield of 4 per cent of the dose, no unchanged N-methylphenobarbital was recovered. They also studied the higher N-alkyl derivatives of barbital to see to what extent dealkylation was responsible for their short action. From the ethyl derivative 30 to 40 per cent of the dose was recovered as barbital. The *n*-propyl derivative in one case yielded a trace of barbital, but usually no barbital could be found. The isopropyl, allyl, *n*-butyl and phenyl derivatives yielded only very small amounts of hypnotic in the urine as determined by bio-assay in mice. Hence, although the inactivation of *n*-butyl and *n*-propylbarbital is even more rapid than that of the methyl and ethyl compounds, the inactivation cannot in any great measure be attributed to dealkylation.

The fact that little or no barbital is found in the urine after the administration of certain N-alkyl derivatives of barbital does not necessarily indicate that dealkylation does not occur, but rather that another reaction is much more rapid. The nature of this other reaction has not been determined. It is known, however, that the introduction of a methyl group on a nitrogen atom of a barbiturate alters the stability of the ring toward aqueous alkali to a degree dependent on the other groups present. It is possible that *in vivo* the rate of ring opening is in some cases more important than that of dealkylation.

Evipal is an N-methylbarbiturate of some clinical importance. Bush and Butler

(12) found that whereas *nor*-Evipal is excreted to the extent of 10 per cent, urine collected after the administration of Evipal contains such small amounts of narcotic material as to indicate that not more than 10 per cent of the drug is demethylated to *nor*-Evipal. Apparently the most important detoxification reaction is oxidation of the cyclohexenyl ring as occurs in Phanodorn and *nor*-Evipal. The same investigators (13) were able to isolate from urine 5-cyclohexenonyl, 5-methylbarbituric acid in an amount equivalent to about 5 per cent of the dose of Evipal. They also isolated two other substances, elementary analyses indicated that they were isomeric 5-cyclohexenonyl, 1,5-dimethylbarbituric acids.

No metabolites of Eunarcon, the N-methyl derivative of Nostal, have been isolated. The drug affords possibilities for demethylation and ring cleavage in addition to the formation of acetonil compounds as in the case of Nostal and Pernoston. The only work reported on this compound is that of Glet (43). He found that urine collected after the administration of Eunarcon gave a negative reaction with the cobalt color test and contained no organic bromine.

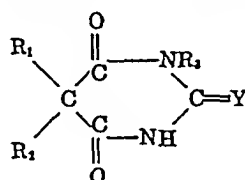
Narconumal is the N-methyl derivative of Alurate. Thalheimer (123) reported that in one human experiment more than 20 per cent of this compound could be recovered after an anesthetic dose of 2 gm. Also, Demole (24) stated that 4 per cent of an intravenous dose of 60 mgm. per kgm. given to a dog was excreted unchanged in the urine in the first 20 hours. If these results are reliable, the entire picture of the metabolism of the barbiturates is complicated further, because they would indicate that the N-methyl-barbituric acids can in certain cases be stable *in vivo*. Unfortunately, neither investigator mentioned his method of identification. Bush and Butler (10) have obtained results which are at variance with those of Thalheimer and of Demole. They found very small amounts of the demethylated compound but no unchanged drug in the urine of dogs anesthetized with Narconumal. In their opinion, the fact that the pharmacological activity in mice was much less than that of Alurate indicated that this compound is different from the other N-methyl compounds and is detoxified in a different manner.

Two thiobarbituric acid derivatives which are of interest clinically are thiopental and Thioethamyl. Apparently these barbiturates are excreted in the urine only in traces (59), but the problem has not received much study. Recently Mark and coworkers (92) reported the isolation of two metabolites of thiopental from urine. They stated that one of these was purified and found to possess at most a mild sedative action. Further details are lacking.

There has been speculation that the sulfur of thiobarbiturates may be replaced by oxygen *in vivo*, which accounts for the prolonged duration of anesthetic effect following large doses. However, there is no chemical evidence to support this. Kozelka and Hine (83) were unable to demonstrate the presence of the sulfur-free analogs in the tissues after successive injections of thiopental and Thioethamyl into dogs and rabbits. Since they were also unable to detect the thiobarbituric acids by color reactions, it seemed probable that the residual depression is due to some metabolic product. Knowing that thiobarbital acts similarly to other barbiturates, Bush and Butler (14) analyzed the urine of dogs treated with this

substance for unchanged thiobarbital and the easily detectable barbital. There was no evidence that either of these substances was present in the urine.

Kemithal, 5-allyl, 5-cyclohexenyl, 2-thiobarbituric acid, has had clinical use in England. Carrington and Raventós (19), using a copper sulfate-diethyl amine color method, found that rabbits given a total of 750 mgm intravenously eliminated about 2 per cent of the dose in the urine. In addition they found substances giving a positive reaction in the cobalt color test equivalent to about 2.5 per cent of the dose. Nearly all these materials were excreted during the first 24 hours following administration. From the urine of a man receiving 6.0 gm of Kemithal by intravenous injection, only 46 mgm of thiobarbituric acid were recovered during the first 24 hours after anesthesia.

GLOSSARY AND INDEX<sup>1</sup>

COMMERCIAL NAME	N.M.R. OR U.S.P. NAME	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Y <sup>2</sup>	PAGE REFERENCES
Allonal (see Alurate)						
Allurate	Aprobarbital	allyl	isopropyl			229, 231, 236
Amytal	Amobarbital	ethyl	isocamyl			220-221, 223-229, 233
Butsol	Butobarbital	ethyl	sec butyl			229
Curral (see Dial)	Vinbarbital	ethyl	1 methyl 1 butenyl			227, 229
Delvinal						
Dial	Diallyl barbituric acid	allyl	allyl			221, 225-229, 231
Dormovit		furfuryl	isopropyl			229, 235
Evipal	Hexobarbital	cyclohexenyl	methyl	methyl		223, 227-229, 230, 234-236
Evipan (see Evipal)						
Eunarcos		2 bromallyl	isopropyl	methyl		229, 236
Ipral	Probarbital	ethyl	isopropyl			229
Kemithal		allyl	cyclohexenyl		S	221, 224, 228, 230, 237
Luminal	Phenobarbital	ethyl	phenyl			219, 224-231, 235
Mebaral (see Prominal)						
Medomin		cycloheptenyl	ethyl			229, 231
Narcosumal		allyl	isopropyl	methyl		229, 236
Nembutal	Pentobarbital	ethyl	1 methylbutyl			221-225, 227-230, 232-233
Neonal	Butethal	n butyl	ethyl			221, 225, 227, 229-232
Noctal (see Noctal)						
Noctal	Propallylonal	2-bromallyl	isopropyl			229, 233-234, 236
Numal (see Alurate)						

COMMERCIAL NAME	N.N.R. OR U.S.P. NAME	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Y <sup>b</sup>	PAGE REFERENCES
Ortal	Hexethal	ethyl	n-hexyl			221, 229, 232
Pentothal	Thiopental	ethyl	1 methylbutyl		B	221, 223-224, 227-228, 230, 235
Pernoston (see Pernoston)						
Pernoston	Butallylonal	2-bromallyl	sec butyl			229-230, 233-234, 235
Phanodorm (see Phanodorm)						
Phanodorm	Cyclobarbital	cyclohexenyl	ethyl			221, 227, 229, 234
Phenitone (see Prominal)						
Prominal		ethyl	phenyl	methyl		229, 235
Rutonal		methyl	phenyl			229, 231
Sandoptal	Allyl barbituric acid	allyl	isobutyl			226, 229, 231
Seconal	Seconal	allyl	1 methylbutyl			224, 227, 229
Sigmodal		2 bromallyl	1 methylbutyl			
Soneryl (see Neonol)						
Surital		allyl	1 methylbutyl		B	228
Thioethamyl		ethyl	isamyl		B	223, 228, 230, 234
Veronal	Barbital	ethyl	ethyl			217-218, 220, 223-230, 233, 237

<sup>1</sup> In this table no distinction is made between barbituric acid derivatives and their salts

<sup>2</sup> R<sub>3</sub> = H unless otherwise designated

<sup>3</sup> Y = O unless otherwise designated.

### SUMMARY

Much published work on the metabolism of the barbiturates cannot be accepted as reliable because of the lack of specificity of the methods of determining the drugs. Colorimetric and ultraviolet spectrophotometric methods are sensitive, but they cannot distinguish the drugs from those metabolic products which also are barbiturates (They are satisfactory only for barbital, which is not degraded *in vivo*). The gravimetric method properly executed gives reliable results, but the sensitivity is not great. Isotope dilution procedures can be both sensitive and specific, but they require considerable special equipment. Pharmacological methods are neither sensitive nor specific.

The absorption, distribution, detoxification and excretion of barbiturates are discussed. It appears that the drugs are rapidly and completely absorbed. Perhaps they vary slightly in the rate at which they enter cells, barbital may be the slowest in this respect. Probably they leave cells readily, as the plasma level falls. The rates of excretion or degradation or both likewise vary and are the more rapid, the shorter the duration of action. Barbital is excreted without change in the urine, as also are appreciable fractions of doses of phenobarbital, Alurate, Dial and Rutonal. Only very small amounts of the other barbiturates escape metabolic alteration which occurs principally but not solely in the liver.

The identification of substances arising from the metabolic degradation of barbiturates is difficult and laborious. The products isolated result from the dealkylation of N-alkyl drugs (e.g., Prominal) or partial oxidation of an alkenyl

or alkyl group in the 5-position (*e g*, Phenodorn, Evipal and pentobarbital) or both (*e g*, Evipal) Drugs containing the 2-bromallyl group (*e g*, Nostal and Pernoston) undergo hydrolysis of the bromine to yield acetonyl derivatives

## REFERENCES

- 1 ANDERSON, B M AND ESSEX, H E Studies on barbiturates especially their cyclic disappearance from and reappearance in the blood following intravenous injection Proc. Staff Meetings Mayo Clinic 17 337-339 1942
- 2 ANDREW, R L AND NEUBAUER, L G The distribution of barbiturates in the human body Analyst, 72, 21-22 1947
- 3 ARON W P, LINEGAR, C R AND DILLE, J M The excretion of barbital in normal and nephropathic subjects J Pharmacol. exper Ther 57 238-263 1936
- 4 BACHM C Das Verhalten des Veronal (Veronal natrum) im Tierkörper bei einmaliger und bei chronischer Darreichung Arch. exper Path. Pharmac., 63 228-241, 1910
- 5 BARRIE, R W AND MAGOUN H W A reducing substance in the urine of cats under Nembutal anesthesia. Proc. Soc. exper Biol Med, 29 684-685, 1932
- 6 BENNHOLD H Über gezielte Transporte verschiedener Medikamente vom Injektionsort zum Erfolgsorgan (Evipal Salvarsan Atebnd Trypanflavin) Verh. dtsch Ges. inn. Med 46 378 379 1934
- 7 BOEDICKER, F AND LUDWIG, H Über Nostal und Pernoston. III Verhalten im Organismus Arch. exper Path. Pharmac., 139 353-356 1929
- 8 BRUNDAOE, J T AND GRUBER C M The determination of barbiturates in the blood and the urine by a new method. J Pharmacol. exper Ther, 59 379-392 1937
- 9 BUSH, M T Hydrolysis of salts of barbituric acids as related to the rate of onset of anesthesia. J Pharmacol. exper Ther., 61: 134-138 1937
- 10 BUSH M T AND BUTLER, T C Metabolic fate of Narconumal (N Methylalurate) J Pharmacol. exper Ther, 66 20 1939
- 11 BUSH M T AND BUTLER, T C The metabolic fate of N substituted derivatives of barbital J Pharmacol. exper Ther., 69: 278-283 1940
- 12 BUSH M T AND BUTLER, T C Metabolic fate of Evipal J Pharmacol. exper Ther, 69: 277 1940
- 13 BUSH M T AND BUTLER, T C The metabolic fate of Evipal J Pharmacol. exper Ther, 72 5 1941
- 14 BUSH M T AND BUTLER, T C Metabolic fate of thiobarbital Fed. Proc., 6 313 1947
- 15 BUTLER, T C AND BUSH, M T The metabolic fate of N methylbarbituric acids J Pharmacol. exper Ther, 65 205-213, 1939
- 16 CAMERON, G R. Some recent work on barbiturates Proc. Royal Soc. Med 32 309-314 1938-39
- 17 CAMERON, G R. COORAY G H. AND DE S N The effect of castration on the action of some barbiturates J Path. Bact 60 239-246 1948.
- 18 CAMERON G R. AND DE SARUM, G S. W The effect of liver damage on the action of some barbiturates J Path. Bact. 43 49-54 1939
- 19 CARRINGTON H C AND RAVENTÓS J Kemithal a new intravenous anesthetic. Brit. J Pharmacol. Chemother., 1: 215-224 1946
- 20 COFFEY R. J, KOFFANYI T AND LINEGAR, C R. The effect of barbiturates on digestive secretion Amer J Dig. Dis., 7 21-23 1940
- 21 COHEN E. L. The determination of barbiturates in toxicological and clinical investigations Am J Pharm 118 40-62 1946
- 22 DAS S C The rates of action and clearance of barbiturates Quart J Exper Physiol, 30 245-248 1940
- 23 DAS S C AND RAVENTÓS J The clearance of sodium Evipal Quart. J Exper Physiol., 29 343, 1939
- 24 DEMOLE V La narcoese par voie intraveineuse. Etude pharmacologique de l'allyl isopropyl n methyl barbiturate de Na (Narconumal Roche) First International Congress of the Union of Therapeutics, Bern 1937, pp 195-204 (Verlag Hans Huber Bern)
- 25 DILLE J M The effect of barbiturates on the embryo and on pregnancy J Pharmacol. exper Ther, 52: 129-136 1934
- 26 DILLE, J M The placental transmission of non anesthetic doses of barbital Amer J Obstet Gynecol, 32, 323-330 1936
- 27 DILLE, J M AND KOFFANYI, T Studies on barbiturates III. Chemical assay of barbiturates J Amer Pharm. Assoc 23 1079-1094 1934
- 28 DILLE, J M LINEGAR, C R AND KOFFANYI T Factors governing the distribution of barbiturates. J Pharmacol. exper Ther 55 46-61 1935
- 29 DORFMAN A AND GOLDBAUM, L. R Detoxification of barbiturates. J Pharmacol. exper Ther 90 330-337 1947
- 30 FARRÉ, R. Etude de la fixation des dérivés barbituriques sur les glandes endocrines C R Soc. Biol., 116 250-281 1934
- 31 FARRÉ, R. AND FREDET P Etude de la localisation et de l'élimination de quelques dérivés alcoylés de la malonylurée J Pharm Chim. 2 321 339 1925
- 32 FARRÉ, R. AND FREDET P Contribution à l'étude de la localisation et de l'élimination de quelques dérivés barbituriques Bull. Soc. Chim. biol 7 1071-1084 1925
- 33 FISCHER, E AND VON MERINO J Ueber Veronal Ther. Gegenwart, 45 145-146 1904.

- 34 FISCHER, P AND KOPPE, J Das Verhalten des Veronals (Veronal Natriums) im menschlichen Körper Münch med Wchnschr 56 1428-1430, 1906
- 35 FISHER, R S, WALKER, J T AND PLUMMER, C W Quantitative estimation of barbiturates in blood by ultra violet spectrophotometry Am J Clin Path, 18 462-469, 1948
- 36 FLEURY, P AND GUINIEBAULT, E L'élimination de la phényldihylmalonylurée C R Soc Biol, 93:1508 1510, 1925
- 37 FRENZ, P AND FARBE, R Etude de la localisation dans l'organisme des dérivés alcoolés de la malonylurée C.R Acad Sci, 180: 469-471, 1925
- 38 FRETWURST F HALBERKANN, J AND REICHE, F Ueber das Allional als Schlafmittel und die Wiederausscheidung seiner Barbitursäurekomponente, des Nemals, durch den Harn Münch med Wchnschr, 77: 1401-1403 1930
- 39 FRETWURST, F, HALBERKANN, J AND REICHE, F, Ueber Dormalgin und Pernocton und über den Abbau ihrer Barbitursäurekomponente im menschlichen Körper Münch med Wchnschr, 77: 1573-1575, 1930
- 40 FRETWURST F, HALBERKANN, J AND REICHE, F Phanodorm und seine Wiederausscheidung mit dem Harn Münch med Wchnschr, 79 1429-1432, 1932
- 41 FRETWURST, F AND NEYER H E Die hypnotische Wirkung und die Ausscheidung des Dormovits Klin Wchnschr, 17: 1372-1374, 1938
- 42 FRETWURST, F, AND VOSS, H, Blut- und Liquorbefunde bei Barbitursäurevergiftungen Klin Wchnschr, 15: 128-129, 1936
- 43 GLET, E Über den Verbleib des Kurzmarkotikums "Eunarcen" im Organismus Klin Wchnschr, 16: 456-458, 1937
- 44 GOLDBAUM L R An ultraviolet spectrophotometric procedure for the determination of barbiturates J Pharmacol exper Ther, 94: 68-76, 1948
- 45 GOULD, T C, MATO E L AND BOWMAN K P Modified ultraviolet spectrophotometric method for quantitative determination of barbiturates Fed Proc, 8 295, 1949
- 46 GREEN, M W, VEITCH F P AND KOPPANYI, T, Studies on barbiturates XXVI The use of Lloyd's reagent in the quantitative estimation of barbiturates in the urine J Amer Pharm Assoc, 32 309-311, 1943
- 47 HALBERKANN, J AND REICHE, F, Ueber die Ausscheidung einiger viel verwandeter Barbitursäureverbindungen mit dem Urin Münch med Wchnschr, 74 1450-1452 1927
- 48 HANDBERG, H Ein neues Prinzip zum Nachweis der Veronalgruppe Kritische Beiträge zur Diagnose der Veronalintoxikation Z ges exper Med, 28 56-80 1922
- 49 HANSON, A Toxicological microanalysis of hypnotics Svensk Kem Tid, 56: 290-294, 1944
- 50 HEFFTER, A Handbuch der experimentellen Pharmakologie Vol I pp 440-448, Julius Springer, Berlin, 1923.
- 51 HEFFTER, A Handbuch der experimentellen Pharmakologie, Ergänzungswerk Vol II, pp 111-153 Julius Springer, Berlin, 1936
- 52 HELLMAN L M, SUETTLER, L B AND STRAN, H A quantitative method for the determination of sodium pentothal in blood J Biol Chem 148 263-267, 1943
- 53 HERWICK, R P A study of barbiturate excretion J Pharmacol exper Ther, 39 267-268, 1930
- 54 HERWICK, R P Further studies on barbiturate excretion J Pharmacol exper Ther 42 268-269, 1931
- 55 HERWICK, R P The determination of barbituric acid derivatives in urine and tissue Arch int Pharmacodyn, 45: 160-166, 1933
- 56 HIRSCHFELDER, A D AND HAURY V G Effect of nephrectomy on duration of action of barbitals Proc Soc Exper Biol Med, 30: 1059-1060, 1933
- 57 HOLCK, H G O, KANAN, M A, MILLS, L M AND SMITH, E L Studies upon the sex-difference in rate in tolerance to certain barbiturates and to nicotine J Pharmacol exper Ther, 60 323-346, 1937
- 58 IVERSEN, M On the serviceability of Koppány's method for quantitative determination of barbituric acid derivatives in urine Acta Pharmacol Toxicol, 1 255-262, 1945
- 59 JAILER J W AND GOLDBAUM L R Studies on the plasma concentration and tissue distribution of sodium Pentothal (sodium ethyl (1 methylbutyl) thiobarbiturate) J Lab Clin Med 31 1344-1349, 1946
- 60 KESER, E Über die Verteilung der Diäthylbarbitursäure im Gehirn Arch exper Path Pharmacol, 186 449-450 1937
- 61 KESER, E AND KESER, I Über die Lokalisation des Veronals, der Phényldiäthyl- und Diäthylbarbitursäure im Gehirn Arch exper Path Pharmacol 125 251-255, 1927
- 62 KESER, E AND KESER, I Über den Nachweis von Coffein Morphin und Barbitursäurederivaten im Gehirn. Arch exper Path Pharmacol, 127 230, 1929
- 63 KESER, E AND KESER, I Die Bedeutung der Sublimation für die Erforschung pharmakologischer Fragen Arch exper Path Pharmacol, 147: 360-365, 1929-30
- 64 KESER, E AND KESER, I Distribution of barbiturates in the brain J Pharmacol exper Ther, 53 137, 1935
- 65 KESER, E AND KESER, I Über die Verteilung der Diäthylbarbitursäure im Gehirn. Arch exper Path Pharmacol, 179: 226-228, 1936
- 66 KELLY, A R AND SHIDMAN, F E Liver as the major organ involved in the detoxication of thiopental by the dog Fed Proc, 8: 306, 1949
- 67 KLIMESCH, K Über die Ursachen der verschieden langen Intervalle zwischen intravenöser Injektion und Wirkung von Schlafmitteln Arch exper Path Pharmacol, 172 10-17, 1933
- 68 KLINOWITZ, M AND REINERT M Die quantitative Bestimmung von Diäthylbarbitursäuren im Harn. Arch exper Path Pharmacol., 165: 418-419 1932
- 69 KOHN, R AND GRIMES, C Studies on the detoxification of sodium pentobarbital under various conditions J Pharmacol exper Ther, 63: 18, 1938



- 70 KOFFANTY, T AND DILLE, J M Experimental analysis of barbital action J Pharmacol exper Ther, 52: 91-100, 1934
- 71 KOFFANTY, T AND DILLE, J M Remarks on the distribution of barbiturates in the brain J Pharmacol exper Ther, 54 84-88 1935
- 72 KOFFANTY, T DILLE J M AND KROP, S Distribution of barbiturates in the brain J Pharmacol exper Ther 52 121-123, 1934
- 73 KOFFANTY, T DILLE J M, MURPHY, W S AND KROP, S Studies on barbiturates II. Contributions to methods of barbital research J Amer Pharm Assoc., 23 1074-1079, 1934
- 74 KOFFANTY T AND KROP S Studies on barbiturates VI The elimination of isomylethyl barbituric acid ("Amytal") and n-butylethyl barbituric acid ("Neonal") J Pharmacol exper Ther, 52 87-90, 1934
- 75 KOFFANTY, T AND LIBERSON, A Studies on the duration of action of drugs I. Analgesics and hypnotics J Pharmacol exper Ther, 39 177-185 1930
- 76 KOFFANTY, T AND LINEGAR, C R Cross-circulation as a method in the study of drug fixation and poisoning Science 96 562-563 1942
- 77 KOFFANTY T, LINEGAR, C R AND DILLE, J M Analysis of the duration of action of barbiturates J Pharmacol exper Ther., 55 62-71 1935
- 78 KOFFANTY, T, MURPHY, W S AND GRAY P L Studies on barbiturates V The action of barbiturates in Sauroptida J Pharmacol exper Ther 52 73-86 1934
- 79 KOFFANTY T, MURPHY W S AND KROP S Studies on barbiturates Arch int Pharmacodyn, 46 76-86, 1933
- 80 KOFFANTY T, MURPHY W S AND KROP, S Colorimetric detection of barbital and its applications Proc Soc Exper Biol Med, 30 542-544 1933
- 81 KOFFANTY T, MURPHY W S AND KROP, S Further contributions to methods of barbital research Proc. Soc Exper Biol Med, 31 373-374 1933
- 82 KOFFANTY, T GREEN M W AND LINEGAR, C R Note on the behavior of sulfonamides in the cobalt color tests for barbiturates J Amer Pharm Assoc, 30 246-247 1941
- 83 KOZELKA F L AND HINE C H Study of the cumulative effects of thiobarbituric acid derivatives J Pharmacol exper Ther., 66 20, 1939
- 84 KOZELKA, F L, NELSON D E AND TATUM H J A method for the determination of barbituric acid derivatives in tissues and body fluids J Pharmacol exper Ther 67 71-78 1939
- 85 KOZELKA, F L AND TATUM, H J A study of the cobalt color reaction for the detection of the barbiturates J Pharmacol exper Ther 59: 54-62 1937
- 86 KOZELKA, F L AND TATUM H J A quantitative study of the barbiturates in cerebrospinal fluid. J Pharmacol exper Ther 59: 63-67, 1937
- 87 KOZELKA F L, TATUM, H J AND NELSON, D E The effect of morphine and liver dysfunction on the distribution and the rate of destruction of isomylethyl barbituric acid (Amytal) J Pharmacol exper Ther, 63: 19, 1938
- 88 KRAUSE, R F AND RILEY R F The reliability of the cobalt-isopropylamine color reaction for Amytal J Pharmacol exper Ther 71 267-272 1941
- 89 LEVY, G A The estimation of barbiturates in the blood Biochem J, 34 73-77, 1940
- 90 LINEGAR, C R, DILLE, J M AND KOFFANTY T Studies on barbiturates XI Further contributions to methods of barbital research J Amer Pharm Assoc. 24 847-852, 1935
- 91 LUNDY, J S AND OSTBERG, A E Review of the literature on the derivatives of barbituric acid Chemistry; pharmacology clinical use. Proc Staff Meetings Mayo Clinic, 4: 386-416 1929
- 92 MARK L C LIEP P A PAPPER, E M, ROVENSTINE, E A, BERNSTEIN, E AND BRODIE, B B Fate of Pentothal in man Fed Proc. 8 318 1949
- 93 MARTIN S J, HERBICH, H C AND CLARK B B The effect of various tissues on the detoxification of Evipal in the dog Anesthesiol, 1: 153-157 1940
- 94 MASON G M C AND BELAND E Influence of the liver and the kidney on the duration of anaesthesia produced by barbiturates Anesthesiol 6 483-491 1945
- 95 MATTIASON K Beiträge zur Kenntnis von der akuten Vergiftung mit Veronal, mit besonderer Bezugnahme auf die Ausscheidung dieses Stoffes mit dem Harn Nord med Ark, 14 Nr 16 (30 pp), 1914
- 96 MAYNERT, E W AND VAN DYKE, H. B The isolation of a metabolite of pentobarbital Science, in press
- 97 MOHR-SCHULZ, W Ueber den toxiologischen Nachweis von Schlafmitteln der Barbitursäurereihe im ärztlichen Laboratorium Münch. med Wchnschr 81 672-673, 1934
- 98 MORRIS, W M The influence of age and sex on the repeated administration of sodium pentobarbital to albino rats. J Pharmacol. exper Ther, 59 68-85 1937
- 99 MUEHL, L. H AND LUNDY, J S. The role of the liver and the kidneys from the standpoint of the anesthetist Anesthesiol 1 40-55, 1940
- 100 MURPHY W S AND KOFFANTY, T Effect of barbiturates in experimental nephrosis J Pharmacol. exper Ther., 52: 70-77, 1934
- 101 PAOET AND DESOYR Dosage. Identification des principaux barbituriques dans l'urine. Etude de leur élimination J Pharm. Chim, 18: 207-214, 1923
- 102 POHL, J Über Allylbarbitursäuren. Z. ges. exper Med., 33: 520-523, 1923
- 103 PRATT T W A comparison of the action of pentobarbital (Nembutal) and sodium barbital in rabbits as related to the detoxicating power of the liver J Pharmacol exper Ther., 48: 235, 1933
- 104 PRATT, T W, VANLANDINGHAM, H W., TALLEY, E E., NELSON, J M AND JOHNSON, E O Studies of the liver function of dogs Amer J Physiol., 102: 148-152, 1932.

- 105 PUCHER, G W The quantitative distribution of Veronal in the organs in the case of Veronal poisoning Bull. Buffalo Gen Hosp, 3 70-71, 1925
- 106 PULVER, R Der chemische Abbau von Medomin im Organismus Schwels med Wehnschr., 73: 124-125, 1943
- 107 PURVES-STEWART, J AND WILLCOX, W Cisternal drainage in coma from barbitone poisoning Lancet, 1: 500-503, 1934
- 108 RAVENTÓS, J Method for the estimation of barbituric and thiobarbituric acids in biological materials. Brit J Pharmacol Chemother, 1: 210-214, 1946
- 109 REICHE F Zur Kenntnis der Veronalvergiftungen und der Veronalausscheidung Klin. Wehnschr, 5: 2112-2113 1926
- 110 REICHE, F AND HALBERKANN, J Ueber Curral und seine Wiederausscheidung mit dem Harn Münch med. Wehnschr, 76 235-236 1929
- 111 REINERT, M Über die Ausscheidung von Barbitursäurederivaten im Harn beim Hund Arch. exper Path. Pharmacol., 130 49-60, 1928
- 112 RILEY, R F, KRAUSE, R F, STEADMAN, L T, HUNTER, F E AND HONOR, H C Color reaction of barbiturates Proc Soc Exper Biol Med, 45 424-427, 1940
- 112a. ROTH, L J, LEIFFER, E, HOONESS, J R AND LANGHAM, W H The metabolism of radioactive pentobarbital in mice J Biol Chem 178 963-966, 1949
- 113 SACK, G Ein neues Verfahren der quantitativen Veronalbestimmung Arch exper Path. Pharmacol, 183 71-76, 1936
- 114 SCHEFFLET C H AND HIGGINS, G M The effect of partial hepatectomy on the action of certain barbiturates and a pheoylurea derivative Amer J Med Sci, 200: 284-268, 1940
- 115 SCHLOSSMAN, H Über die Ausscheidung von Veronal und Quadronox Arch exper Path Pharmacol, 173 129-132, 1933
- 116 SHAW, F H The estimation of barbituric acid derivatives Australian J Exper Biol Med Sci, 20: 47-48, 1942
- 117 SHIDEMAN F E KELLY, A R AND ADAMS, B J The role of the liver in the detoxication of thiopental (Pentothal) and two other thiobarbiturates J Pharmacol exper Ther, 91: 331-339, 1947
- 118 SHONLE, H A, KILICH A K, KESFF, G F AND SWANSON, E E The question of elimination of barbituric acid derivatives in the urine with special reference to isomyl ethyl barbituric acid (sodium Amytal) and 1-methylbutyl ethyl barbituric acid (pentobarbital sodium) J Pharmacol exper Ther, 49: 393-407, 1933
- 119 STARKENSTEIN, E AND KLINGSCH K Über die pharmakologische Wirkung von Additionsverbindungen und Gemengen Arch exper Path Pharmacol, 176 494-503 1934
- 120 TATUM, A L The present status of the barbiturate problem Physiol Rev, 19 472-502, 1939
- 121 TATUM, A L The pharmacology of barbiturates Ann Rev Physiol, 2 359-370 1940
- 122 TATUM, H J NELSON D E AND KOZELKA, F L A study of the effects of morphine and of carbon tetrachloride on the rate of disappearance of ethyl isomyl barbituric acid J Pharmacol exper Ther, 72 123-129, 1941
- 123 THALHEIMER, M Anesthésie par injections intraveineuses d'acide 1 méthyl 5 5 allyl iso-propyl barbiturique Anesth Analg 2 560-570, 1936
- 124 TYSON, R M SHRADES E A AND PERLMAN, H H Drugs transmitted through breast milk II Barbiturates J Pediatr, 13 88-90 1938
- 125 VAN DYKE, H B, SCUDI J V AND TARKEN D L The excretion of Nu in the urine of dogs after the administration of labeled pentobarbital J Pharmacol exper Ther, 90 364-366, 1947
- 126 VOOR M Die Verteilung von Arzneistoffen auf verschiedene Regionen der Zentralnervensystems, zugleich ein Beitrag zu ihrer quantitativen Mikrobestimmung in Gewebe V Mitteilung Barbitursäurederivate Arch exper Path Pharmacol, 178 601-627, 1935
- 127 WALKER J T, FISHER R S AND McHUON, J J Quantitative estimation of barbiturates in blood by ultra violet spectrophotometry I Analytical method Am J Clin Path, 18 461-461, 1948
- 128 WEESE, H Moderne Schlafmitteltherapie und Basisnarkose Arch exper Path Pharmacol, 181: 46-61, 1936
- 129 WEESE, H Thérapeutique moderne par les hypnotiques et la narcose de base Anesth Analg, 2: 12-40 1936
- 130 WERNER, H W, PRATT, T W AND TATUM, A L A comparative study of several ultra short acting barbiturates, Nembutal, and tribromethanol J Pharmacol exper Ther, 60: 189-197 1937
- 131 WORTCHALL, R AND WHELEO HILL Über die Ausscheidung des Nektals Dtsch med Wehnschr, 54 140-141, 1928
- 132 ZWIKKER, J J L Het Aantoonen en het Afsonderen van Barbitalen bij het toxicologisch Onderzoek Pharm. Weekblad, 69 975-983, 1931, Recherches et isolement des barbitales dans les expertises toxicologiques J Pharm Chim, 19: 189 1934
- 133 ZWIKKER, J J L AND STERNHAVER A J The distribution of barbital in the brain in a case of acute barbital poisoning Cited by Chem Abstr., 34: 4158 1940

## SPINAL CORD DEPRESSANT DRUGS\*

F M BERGER, M.D †

*The Department of Pediatrics, The University of Rochester School of Medicine and Dentistry, Rochester, New York*

The pharmacology of drugs possessing a depressant action on the spinal cord received little attention in the past. The discovery of useful applications of curare in medicine and surgery directed attention to other substances capable of producing paralysis. The purpose of the present article is to summarize the pharmacological properties of various substances producing paralysis of skeletal muscles by a depressant action on the spinal cord. Because of the lack of suitable laboratory methods for the evaluation of the therapeutic potentialities of these substances, the clinical results obtained with some of them have also been briefly reviewed. The actions of local anesthetic drugs on the spinal cord were specifically omitted.

### TRI-*O*-CRESYL PHOSPHATE

During the early part of 1930 a large number of people in the southwestern part of the United States developed a peculiar form of paralysis which appeared about 10 days after the consumption of an alcoholic beverage sold as "Fluid extract of Jamaica Ginger U S P" and often called "Jake." Smith *et al* (113, 114) have shown that tri-*o*-cresyl phosphate, present as an adulterant in certain fluid extracts of Jamaica ginger, was the etiologic agent of the epidemic of ginger paralysis.

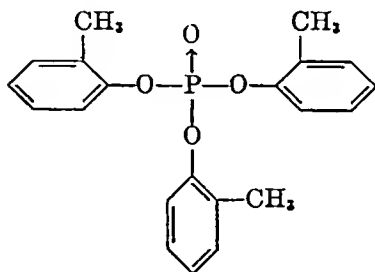


FIG 1 Structural formula of tri-*o*-cresyl phosphate

**Clinical course** The disability began with soreness of the leg muscles which was soon followed by bilateral foot drop. Bilateral wrist drop also developed in many cases but the disability in the hands was never as marked as in the feet. Paralysis was always bilateral and symmetrical. The clinical picture indicated an involvement of the lower motor neuron localized to the lower lumbar and lower cervical regions of the spinal cord.

\* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

† Present address: Wallace Laboratories, Inc., New Brunswick, New Jersey

*Effects in animals* Tri-*o*-cresyl phosphate administered orally to rabbits or guinea pigs in doses of 50–100 mg per kg caused at first a mild degree of hyperexcitability with generalized muscular tremor and incoordination. This state passed after hours or days, depending on the dose given, into a picture dominated by flaccid muscular asthenia and generalized flaccid paralysis. The rabbits did not show symptoms comparable to the characteristic wrist drop and foot drop observed in human beings after ingestion of adulterated ginger extract. The symptoms of tri-*o*-cresyl phosphate poisoning in the rabbit were, however, exactly the same as those observed after administration of adulterated ginger extracts to these animals.

Oral administration of tri-*o*-cresyl phosphate to dogs, cats and monkeys was without effect but subcutaneous or intramuscular injections produced motor paralysis of the hind limbs after an interval of 6 or more days. In calves and chickens, paralysis similar to that observed in humans could be produced by both oral and parenteral administration (113). Albino rats appeared wholly refractory to the effects of the poison by all routes of administration. Mice were relatively insensitive, flaccid paralysis of the hind legs occurred in some animals after large doses (68).

*The site of action* The pharmacological examination of the neuromuscular apparatus in tri-*o*-cresyl phosphate poisoning in hens (115) indicated that neither the muscle fibres nor the motor end plates were affected. Histological examination of the nervous system in Jake paralysis in man and tri-*o*-cresyl poisoning in various animals showed degeneration of the myelin sheaths of the peripheral nerves and degenerative changes of the anterior horn cells throughout the spinal cord (116). Autonomic cells of the cord and dorsal root ganglia also showed definite abnormalities (76).

*Effect on enzymes* Bloch (23) found that tri-*o*-cresyl phosphate had a marked inhibiting action on cholinesterase and serum lipase *in vitro* and *in vivo*. Tri-*m*-cresyl phosphate and tri-*p*-cresyl phosphate did not inhibit these enzymes. The action of the *o*-isomer was not due to the liberation of *o*-cresol in the animal body.

If acetylcholine plays an important role in the transmission of impulses in the spinal cord, a relationship between the anticholinesterase activity and the paralyzing property of tri-*o*-cresyl phosphate might be expected. However, other substances possessing an inhibiting action on cholinesterase do not produce paralysis. It is possible that the action of tri-*o*-cresyl phosphate on other serum esterases may play an important part in the production of ginger paralysis.

*Action of related compounds* Tri-*p*-cresyl phosphate and tri-*m*-cresyl phosphate were much less toxic than the *o*-isomer and failed to produce toxic effects in rabbits, chickens and cats in doses up to 3 grams per kg. Triphenyl phosphate, phenol and the three isomeric cresols were also considerably less toxic than tri-*o*-cresyl phosphate and differed from the latter by the production of symptoms soon after administration of toxic dose (114, 115). Tri-*o*-cresyl phosphite produced qualitatively similar symptoms as the phosphate in rabbits, chickens and rats. In cats, flaccid paralysis or marked extensor rigidity, particularly of the hind limbs, was

observed (115) The histological lesions produced by tri-*o*-cresyl phosphite consisted in a degeneration of the ascending spino-cerebellar tracts and the descending mesencephalic-pontine-cerebello-spinal tracts In addition, there was also minor degeneration of the lower motor neuron characteristic of tri-*o*-cresyl phosphate (79)

*Industrial and medicinal use of tri-*o*-cresyl phosphate* Tri-*o*-cresyl phosphate and mixtures of the three isomers are widely used in industry as plasticizers in the manufacture of celluloid, paints and varnishes and in tanning of leather Hodge and Sterner (68) examined the skin absorption of tri-*o*-cresyl phosphate with the help of radioactive phosphorus They found that absorption through the palmar skin of the hands may occur and may constitute a real hazard in industrial operations permitting repeated exposures to this compound

Polyn neuritis has followed the use of an abortifacient known as Apiol which contains tri-*o*-cresyl phosphate (72a) Certain cases of polyn neuritis and of acute ascending paralysis of the Landry type may be due to tri-*o*-cresyl phosphate poisoning (76) Transient paresis of the legs in all members of a family which used a butter substitute and salad oil containing tri-*o*-cresyl phosphate has been described (58a)

#### DITHIOBIURET

During studies concerning the antithyroid activity of compounds related to thiourea, Astwood *et al* (2) observed that the chronic administration of dithiobiuret to rats caused reversible paralysis of the skeletal muscles

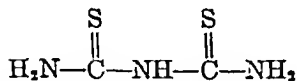


FIG 2 Structural formula of dithiobiuret

*Effect on animals* When dithiobiuret was given in a single dose, it either did not produce symptoms or caused death, the effect observed depending on the amount of the drug given The lethal dose of the compound for an adult rat from a single injection was 20 to 50 mg On chronic administration of the drug in drinking water in concentrations of 0 001 per cent to 0 002 per cent, corresponding to less than 0 5 mg of the drug per rat per day profound muscular paresis was observed after 2 to 4 days Paralysis appeared first in the hind legs and later ascended to affect all voluntary muscles of the body with the exception of those of respiration, the head and neck Paralysis was not complete as the animals were able to feed and water themselves Muscular paresis was maintained as long as the administration of the drug was continued Animals which had been paralysed for some time lost weight and showed wasting and contractures of the paralysed muscles Discontinuation of the drug resulted in complete recovery of muscle function in a few days The chronic administration of slightly larger doses of dithiobiuret caused muscular paralysis and death by respiratory paralysis at the end of 5 to 6 days

*The site of action* The site of action of dithiobiuret is in the central nervous system. Faradic stimulation of motor nerves elicited muscular contraction in paralysed animals. The fact that strychnine failed to induce convulsions in paralysed animals suggests that dithiobiuret possesses a selective depressant action on the spinal cord (2). Altschul (1) did not observe any changes in the electroencephalograms of animals tested after acute or prolonged administration of dithiobiuret.

*The anticonvulsant action* In rats, dithiobiuret raised the threshold to electrically induced convulsions and modified the form of convulsions. While normal animals showed tonic-clonic seizures, animals under the influence of dithiobiuret showed seizures of sustained tonic character followed by only minimal clonic movement (1). These observations are rather interesting in view of the findings of Toman *et al* (121) who have shown that anticonvulsant drugs as a rule abolish the extensor tonic component of the maximal seizure pattern in doses which do not influence or even prolong the clonic phase. Dithiobiuret, therefore, appears to possess a qualitatively different anticonvulsant action. However, the anticonvulsant effect of dithiobiuret was well marked only in animals which were paralysed (1).

*The mode of action* Histological examination of the central nervous system of animals paralysed with dithiobiuret did not disclose any structural damage. The acetylcholine and cholinesterase content of brains of paralysed rats was normal. Paralysis could not be counteracted by the administration of pilocarpine, neostigmine, atropine or epinephrine or by large doses of crude liver extract, thiamine, nicotinic acid, vitamin A, biotin, brewer's yeast or biuret (2). Astwood *et al* (2) believe that dithiobiuret interferes with a hitherto unrecognized process essential to the transmission of impulses in the central nervous system, by blocking a component of some enzyme system.

#### BENZIMIDAZOLE

Goodman, Gilman and Hart (51, 49) in 1943 made the interesting observation that the simple chemical compound benzimidazole caused transient paralysis in various species of laboratory animals.

*The paralyzing action* An intraperitoneal injection of benzimidazole to mice or cats in doses of 200 to 300 mg per kg caused a profound decrease of muscle tone and loss of postural reflexes. After administration of the drug, the hind legs became affected first, the trunk and foreleg muscles next and the neck musculature was the last to show the effect of the drug. Large doses of the drug caused complete muscular paralysis which appeared in the order mentioned. During paralysis respiration remained adequate and was sometimes increased during the initial phase of action of the compound.

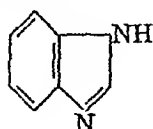


FIG 3 Structural formula of benzimidazole

During paralysis, corneal and pupillary reflexes were normal and deep reflexes normal or exaggerated. There was little response to painful stimuli, but the impairment was probably secondary to the spinal cord deficit, since very painful stimuli caused dilatation of the pupil.

Paralysis lasted for about one hour and was followed by gradual recovery of muscular power. Recovery as a rule was complete about 4 hours after administration of the drug. Paralysis was not preceded by excitation and was not accompanied by a loss of consciousness. These observations indicate that benzimidazole can produce selective cord paralysis in doses which do not cause anesthesia.

*Effect on vital functions and toxicity* Electrocardiographic studies showed that the heart was uninfluenced by larger doses of benzimidazole. The fall of blood pressure observed after rapid intravenous injection of the compound may have been due to vasodilatation.

Respiration remained adequate and the animals continued to breathe spontaneously even during complete paralysis of all voluntary muscles. Death from toxic doses was due to respiratory paralysis. In mice and probably also in other animals the mean lethal dose was more than twice as large as the mean paralyzing dose. Benzimidazole, therefore, possessed an adequate margin of safety.

Chronic administration of benzimidazole for many weeks to rats and mice in doses which did not affect voluntary movements, did not influence the growth, behaviour or general appearance of the animals. No gross changes were observed in any organs after the animals were sacrificed.

Benzimidazole has been effectively administered by the intravenous, intraperitoneal, subcutaneous, and oral routes. The compound is apparently well absorbed. Data concerning the fate of the compound in the body are not available. It appears probable that it is rapidly inactivated in the body as cumulative toxicity did not develop. Administration continued over long periods of time did not increase the susceptibility of the animals to the effect of the drug.

*Actions on the nervous system* Paralyzing doses of benzimidazole did not exert any effect on peripheral nerves and did not affect transmission at the neuromuscular junction. Although full doses of benzimidazole did not cause loss of consciousness and did not affect the electroencephalographic patterns, the drug did elevate the thresholds for evoked cortical potentials and seizures. The loss of the postural reactions and the ascending type of paralysis are not incompatible with a depressant action of the drug on the midbrain and spinal cord (49). During paralysis the deep reflexes were exaggerated and clonus was sometimes present. The specific structure in the central nervous system on which this depressant action is exerted may well be the interneurons. This view is supported by the finding that benzimidazole has a depressant action on multineuron reflexes in the spinal cord, such as the flexor or crossed extensor reflexes, but has little effect on the knee jerk which is mediated by a two-neuron arc (53). The transient emesis sometimes observed after intravenous administration of benzimidazole may be due to a depressant action on inhibitory interneurons of the vomiting center.

The effect of benzimidazole in animals in which various ablations were carried out is of interest. In acute decerebrated cats, benzimidazole caused a disappear-

*The site of action* The site of action of dithiobiuret is in the central nervous system. Faradic stimulation of motor nerves elicited muscular contraction in paralysed animals. The fact that strychnine failed to induce convulsions in paralysed animals suggests that dithiobiuret possesses a selective depressant action on the spinal cord (2). Altschul (1) did not observe any changes in the electroencephalograms of animals tested after acute or prolonged administration of dithiobiuret.

*The anticonvulsant action* In rats, dithiobiuret raised the threshold to electrically induced convulsions and modified the form of convulsions. While normal animals showed tonic-clonic seizures, animals under the influence of dithiobiuret showed seizures of sustained tonic character followed by only minimal clonic movement (1). These observations are rather interesting in view of the findings of Toman *et al* (121) who have shown that anticonvulsant drugs as a rule abolish the extensor tonic component of the maximal seizure pattern in doses which do not influence or even prolong the clonic phase. Dithiobiuret, therefore, appears to possess a qualitatively different anticonvulsant action. However, the anticonvulsant effect of dithiobiuret was well marked only in animals which were paralysed (1).

*The mode of action* Histological examination of the central nervous system of animals paralysed with dithiobiuret did not disclose any structural damage. The acetylcholine and cholinesterase content of brains of paralysed rats was normal. Paralysis could not be counteracted by the administration of pilocarpine, neostigmine, atropine or epinephrine or by large doses of crude liver extract, thiamine, nicotinic acid, vitamin A, biotin, brewer's yeast or biuret (2). Astwood *et al* (2) believe that dithiobiuret interferes with a hitherto unrecognized process essential to the transmission of impulses in the central nervous system, by blocking a component of some enzyme system.

#### BENZIMIDAZOLE

Goodman, Gilman and Hart (51, 49) in 1943 made the interesting observation that the simple chemical compound benzimidazole caused transient paralysis in various species of laboratory animals.

*The paralyzing action* An intraperitoneal injection of benzimidazole to mice or cats in doses of 200 to 300 mg per kg caused a profound decrease of muscle tone and loss of postural reflexes. After administration of the drug, the hind legs became affected first, the trunk and foreleg muscles next and the neck musculature was the last to show the effect of the drug. Large doses of the drug caused complete muscular paralysis which appeared in the order mentioned. During paralysis respiration remained adequate and was sometimes increased during the initial phase of action of the compound.

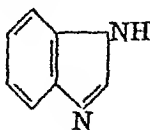


FIG. 3 Structural formula of benzimidazole



During paralysis, corneal and pupillary reflexes were normal and deep reflexes normal or exaggerated. There was little response to painful stimuli, but the impairment was probably secondary to the spinal cord deficit, since very painful stimuli caused dilatation of the pupil.

Paralysis lasted for about one hour and was followed by gradual recovery of muscular power. Recovery as a rule was complete about 4 hours after administration of the drug. Paralysis was not preceded by excitation and was not accompanied by a loss of consciousness. These observations indicate that benzimidazole can produce selective cord paralysis in doses which do not cause anesthesia.

*Effect on vital functions and toxicity* Electrocardiographic studies showed that the heart was uninfluenced by larger doses of benzimidazole. The fall of blood pressure observed after rapid intravenous injection of the compound may have been due to vasodilatation.

Respiration remained adequate and the animals continued to breathe spontaneously even during complete paralysis of all voluntary muscles. Death from toxic doses was due to respiratory paralysis. In mice and probably also in other animals the mean lethal dose was more than twice as large as the mean paralyzing dose. Benzimidazole, therefore, possessed an adequate margin of safety.

Chronic administration of benzimidazole for many weeks to rats and mice in doses which did not affect voluntary movements, did not influence the growth, behaviour or general appearance of the animals. No gross changes were observed in any organs after the animals were sacrificed.

Benzimidazole has been effectively administered by the intravenous, intraperitoneal, subcutaneous, and oral routes. The compound is apparently well absorbed. Data concerning the fate of the compound in the body are not available. It appears probable that it is rapidly inactivated in the body as cumulative toxicity did not develop. Administration continued over long periods of time did not increase the susceptibility of the animals to the effect of the drug.

*Actions on the nervous system* Paralyzing doses of benzimidazole did not exert any effect on peripheral nerves and did not affect transmission at the neuromuscular junction. Although full doses of benzimidazole did not cause loss of consciousness and did not affect the electroencephalographic patterns, the drug did elevate the thresholds for evoked cortical potentials and seizures. The loss of the postural reactions and the ascending type of paralysis are not incompatible with a depressant action of the drug on the midbrain and spinal cord (49). During paralysis the deep reflexes were exaggerated and clonus was sometimes present. The specific structure in the central nervous system on which this depressant action is exerted may well be the interneurons. This view is supported by the finding that benzimidazole has a depressant action on multineuron reflexes in the spinal cord, such as the flexor or crossed extensor reflexes, but has little effect on the knee jerk which is mediated by a two-neuron arc (53). The transient emesis sometimes observed after intravenous administration of benzimidazole may be due to a depressant action on inhibitory interneurons of the vomiting center.

The effect of benzimidazole in animals in which various ablations were carried out is of interest. In acute decerebrated cats, benzimidazole caused a disappear-

*The site of action* The site of action of dithiobiuret is in the central nervous system. Faradic stimulation of motor nerves elicited muscular contraction in paralysed animals. The fact that strychnine failed to induce convulsions in paralysed animals suggests that dithiobiuret possesses a selective depressant action on the spinal cord (2). Altschul (1) did not observe any changes in the electroencephalograms of animals tested after acute or prolonged administration of dithiobiuret.

*The anticonvulsant action* In rats, dithiobiuret raised the threshold to electrically induced convulsions and modified the form of convulsions. While normal animals showed tonic-clonic seizures, animals under the influence of dithiobiuret showed seizures of sustained tonic character followed by only minimal clonic movement (1). These observations are rather interesting in view of the findings of Toman *et al* (121) who have shown that anticonvulsant drugs as a rule abolish the extensor tonic component of the maximal seizure pattern in doses which do not influence or even prolong the clonic phase. Dithiobiuret, therefore, appears to possess a qualitatively different anticonvulsant action. However, the anticonvulsant effect of dithiobiuret was well marked only in animals which were paralysed (1).

*The mode of action* Histological examination of the central nervous system of animals paralysed with dithiobiuret did not disclose any structural damage. The acetylcholine and cholinesterase content of brains of paralysed rats was normal. Paralysis could not be counteracted by the administration of pilocarpine, neostigmine, atropine or epinephrine or by large doses of crude liver extract, thiamine, nicotinic acid, vitamin A, biotin, brewer's yeast or biuret (2). Astwood *et al* (2) believe that dithiobiuret interferes with a hitherto unrecognized process essential to the transmission of impulses in the central nervous system, by blocking a component of some enzyme system.

#### BENZIMIDAZOLE

Goodman, Gilman and Hart (51, 49) in 1943 made the interesting observation that the simple chemical compound benzimidazole caused transient paralysis in various species of laboratory animals.

*The paralyzing action* An intraperitoneal injection of benzimidazole to mice or cats in doses of 200 to 300 mg per kg caused a profound decrease of muscle tone and loss of postural reflexes. After administration of the drug, the hind legs became affected first, the trunk and foreleg muscles next and the neck musculature was the last to show the effect of the drug. Large doses of the drug caused complete muscular paralysis which appeared in the order mentioned. During paralysis respiration remained adequate and was sometimes increased during the initial phase of action of the compound.

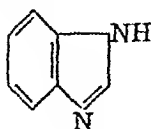


FIG 3 Structural formula of benzimidazole

*The mode of action* The mode of action of benzimidazole is not known. It appears probable that benzimidazole blocks an essential metabolite or enzyme necessary for the propagation of impulses through the interneurons. The action of benzimidazole is in some respects similar to that of myanesin and glyketal and it appears possible that all three drugs have a similar mode of action. Benzimidazole, however, differs from myanesin in affecting cortical mechanisms to a lesser extent (118a).

*Related compounds* Goodman (49) examined a number of congeners of benzimidazole, including the methyl, phenyl and dibenzimidazole derivatives. None approached benzimidazole in potency and deviations from the parent structure resulted in loss of the characteristic pharmacodynamic properties.

#### MYANESIN AND OTHER GLYCEROL ETHERS

Gilbert and Descomps (47) in 1910 observed that 3-phenoxypropane-1,2-diol caused transient paralysis of skeletal muscles in guinea pigs and rabbits. They also noted the antipyretic and local anesthetic properties of this substance and recommended its use in humans as an analgesic and antipyretic. It became commercially available under the name of Antodyne (44). The findings of the French authors were soon confirmed and amplified by Filippi and Rodolico (42). Berger and Bradley (16) examined the pharmacological properties of numerous simple mono ethers of glycerol and found that most of these compounds had a qualitatively similar action. Myanesin, 3-(2'-methylphenoxy) propane-1,2-diol, one of the more potent compounds of the series, has since been the subject of numerous pharmacological and clinical studies and has been made commercially available under various names (Lissephen, Oranixon, Tolserol and Toloxyn in the United States, Myanesin in Great Britain and the British Commonwealth, Relaxar in France (77) and Glycresin in northern Europe (22)).

Short reviews on myanesin have been published (130, 40).

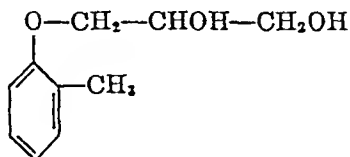


FIG. 4 Structural formula of myanesin

*Physical and chemical properties* Myanesin is a white, crystalline solid, and has a melting point of 70–71°C. It is odorless, has a bitter taste and produces a sensation of numbness on the tongue. Solubility in water at 20°C is 1 in 85, in 10 per cent urethane solution, 1 in 40, and in 25 per cent urethane solution, 1 in 4.5. It is very soluble in ethyl alcohol, propylene glycol and aqueous solutions of urea and ethyl urea. Relatively stable supersaturated aqueous solutions can be obtained by cooling solutions prepared at higher temperatures. Solutions of the drug are stable and can be sterilized by heating, and are compatible and misci-

ance of extensor rigidity and of tonic neck and labyrinthine reflexes. In acute decorticate or hypothalamic preparations, sham rage was abolished and, in spinal cats, responses to nociceptive stimuli disappeared but myotatic reflexes were enhanced (49, 52). The spastic syndrome in a monkey with bilateral resection of cortical area 6 as well as bilateral removal of the caudate nucleus was improved by benzimidazole (51).

*The anticonvulsant action.* The effects of benzimidazole could be antagonized by mephenthamide (52) and metrazol (49). Benzimidazole also decreased the incidence and severity of metrazol convulsions in mice. In animals in which multi-neuron reflexes have been depressed by benzimidazole, metrazol was less effective than mephenthamide in antagonizing these effects on the cord (119). Benzimidazole possessed a powerful antagonistic effect to convulsions and death produced by strychnine and was in this respect at least equal and probably superior to myanesin (7).

Benzimidazole abolished the extensor tonic component of maximal electroshock seizures in rabbits, cats and rats (120). It shared this property with clinically effective antiepileptic drugs such as diphenylhydantoin and phenobarbital, but was considerably less specific in its action.

*The diuretic action.* Rats given daily doses of benzimidazole 200 mg per kg intraperitoneally exhibited marked polydipsia and polyuria. Polyuria was detectable after 3 days and persisted as long as benzimidazole was administered. Despite a 10 to 20 fold increase in urinary volume, young rats continued to grow and maintained chloride balance. There were no histopathologic changes in the hypothalamus, posterior pituitary, kidneys or other organs. Polyuria was not influenced by large doses of posterior pituitary hormone and was probably due to a specific inhibition of renal tubular reabsorption of water (52).

*Other pharmacological properties.* Benzimidazole was devoid of local anesthetic action. It had no spasmolytic effect on smooth muscle (49). Wooley (134a) found that benzimidazole in concentrations of 600 micrograms per cc of medium completely inhibited the growth of *Saccharomyces cerevisiae*. Half maximum inhibition of growth with this organism, *E. coli* and *S. lactis R* was obtained with concentrations of 300 micrograms per cc, 370 micrograms per cc and 725 micrograms per cc, respectively. Adenine (1000 micrograms per cc) and guanine reversed inhibition but other purines had little or no effect. The paralyzing action of benzimidazole was not prevented by adenine.

*Clinical use.* Goodman (49) suggested that benzimidazole, because of its effect on skeletal muscle tone and its anticonvulsant action, may be of value in the treatment of spastic and convulsant disorders. He also considered the use of the drug as a supplemental agent in general anesthesia to increase muscular relaxation. As benzimidazole is readily soluble in water, it may be more suitable for this purpose than the relatively insoluble myanesin. Benzimidazole given intravenously in small dosage to a spastic patient caused a decrease of muscle tone, some nausea and evidence of early intercostal involvement (50). In another patient suffering from Little's disease temporary relaxation of skeletal muscle spasm was observed (52).

animals Thus the mean paralysing doses for mice, rats, rabbits and dogs on intravenous administration were approximately 150, 110, 50 and 30 mg per kg, respectively This relation between the size of the dose and size of the animal species is not unusual and holds true for many drugs

After rapid intravenous injection of myanesin to rabbits, transient rigidity was sometimes observed (9, 28) The rigidity was similar to that observed after decerebration and was probably caused by a "pharmacological transection" due to the depressant action of the drug on certain structures of the midbrain and not by a direct action on skeletal muscle The rigidity was transient and was followed by complete flaccid paralysis

Guinea pigs appeared somewhat less sensitive to the paralysing effect of myanesin than other species and frequently showed salivation, dyspnoea and ruffled fur following administration of the drug (7)

TABLE 1

*Mean paralysing (PD<sub>50</sub>) and mean lethal doses (LD<sub>50</sub>) in mice and rats after administration of myanesin by various routes*

ROUTE		MICE	RATS
Intravenous	PD <sub>50</sub> mg/kg	150 ± 6	113 ± 10
	LD <sub>50</sub> mg/kg	322 ± 11	195 ± 10
Intraperitoneal	PD <sub>50</sub> mg/kg	178 ± 9	120 ± 10
	LD <sub>50</sub> mg/kg	610 ± 10	430 ± 18
Subcutaneous	PD <sub>50</sub> mg/kg	325 ± 20	
	LD <sub>50</sub> mg/kg	1000 ± 56	
Oral	PD <sub>50</sub> mg/kg		1330 ± 80
	LD <sub>50</sub> mg/kg		2150 ± 148

In frogs (*R. temporaria*) flaccid paralysis and cessation of respiration were obtained after 3 to 10 mg of myanesin injected into the anterior lymph sac In frogs myanesin also caused a loss of indirect excitability of the muscle (curare-like action) in doses which caused reversible paralysis In this respect the action of the drug in frogs differed from that in mammals in which loss of indirect excitability of muscle after tolerated doses of myanesin was not observed (16)

The injection of myanesin into the cavity of marine bivalves caused prompt relaxation of the constrictor muscle and opening of the shell (7)

*Acute toxicity* Death from toxic doses was due to respiratory paralysis The heart continued beating for a short time after respiration had ceased Table 1 gives the mean paralysing and mean lethal doses obtained after administration of myanesin by various routes to mice and rats It shows that there is an adequate margin of safety between paralysing and lethal doses of the drug The standard safety margin of myanesin calculated according to Foster (43) after intraperitoneal administration to mice was 113 per cent

Post mortem examinations carried out in animals dying after large doses of myanesin showed moderate engorgement of the liver and spleen, subpleural hemorrhages in the lungs and distention of the right auricle with blood (7)

ble with solutions of sodium chloride, glucose, barbiturates and thiobarbiturates (16, 17)

A solution of 0.01 g of myanesin in 10 drops of concentrated sulphuric acid is slightly red, on addition of a drop of formaldehyde solution a deep red color appears (93)

*The paralyzing action.* Small doses of antodyne or myanesin caused reduction of spontaneous activity and a decrease in muscle tone. Larger doses produced ataxia, flaccid paralysis and loss of the righting reflex. Muscular paralysis was always of the ascending type. The posterior limbs and the lower half of the body were affected first and remained paralysed longer than the anterior limbs and neck muscles (42, 16). Respiration was not embarrassed even during complete paralysis of skeletal muscles. During the initial phase of the drug action, respiration was sometimes increased in depth and rate. Paralysis was neither preceded nor followed by excitation or convulsions and was followed by complete recovery of muscular power.

Paralysis occurred within 2 minutes after administration of the drug. The depth and duration of paralysis depended on the amount of drug given. In mice after intraperitoneal administration of 250 mg of myanesin per kg, the righting reflex was lost for about 25 minutes. During paralysis the animals remained motionless and did not execute running movement usually observed after administration of small doses of barbiturates or other anesthetics. The animals were completely limp and lacked righting reflexes. They reacted with powerful and sustained muscular contractions to painful stimuli. The pupillary and corneal reflexes and the knee jerk were unchanged. There was no change in the size of the pupils.

During the peak of paralysis after large doses of the drug, the corneal reflex was lost, the pupils were somewhat dilated and reacted sluggishly to light. Nystagmus was observed for short periods of time during the peak of the drug action. There was some dilatation of the pupil in response to nociceptive stimuli but the withdrawal reflex was abolished. Large doses of myanesin caused salivation in rabbits and cats but vomiting was not observed.

Recovery from paralysis was usually rapid. There was some incoordination of movements for about one hour after muscular power was regained. Burke and Linegar (28) observed nausea and vomiting during recovery from paralysis in dogs.

*Route of administration.* Myanesin could be effectively administered to animals by the intravenous, intraperitoneal, intramuscular, subcutaneous, rectal and oral routes. The amount of drug producing paralysis varied greatly with the route of administration (Table 1). On intravenous administration, the effect obtained varied greatly with the speed of injection. In rabbits the largest tolerated dose on rapid injection was about 100 mg per kg whereas as much as 350 mg per kg could be tolerated if the injection was carried out very slowly (16).

*Species sensitivity.* Myanesin produces paralysis in most species of laboratory animals. When the amount of drug required for the production of paralysis was expressed in mg per kg body weight, smaller doses of myanesin were required for the production of paralysis in species of large animals than in species of small

animals. Thus the mean paralysing doses for mice, rats, rabbits and dogs on intravenous administration were approximately 150, 110, 50 and 30 mg per kg, respectively. This relation between the size of the dose and size of the animal species is not unusual and holds true for many drugs.

After rapid intravenous injection of myanesin to rabbits, transient rigidity was sometimes observed (9, 28). The rigidity was similar to that observed after decerebration and was probably caused by a "pharmacological transection" due to the depressant action of the drug on certain structures of the midbrain and not by a direct action on skeletal muscle. The rigidity was transient and was followed by complete flaccid paralysis.

Guinea pigs appeared somewhat less sensitive to the paralysing effect of myanesin than other species and frequently showed salivation, dyspnoea and ruffled fur following administration of the drug (7).

TABLE 1

*Mean paralysing (PD<sub>50</sub>) and mean lethal doses (LD<sub>50</sub>) in mice and rats after administration of myanesin by various routes*

ROUTE		MICE	RATS
Intravenous	PD <sub>50</sub> mg/kg	150 ± 6	113 ± 10
	LD <sub>50</sub> mg/kg	322 ± 11	195 ± 10
Intraperitoneal	PD <sub>50</sub> mg/kg	178 ± 9	120 ± 10
	LD <sub>50</sub> mg/kg	610 ± 10	430 ± 18
Subcutaneous	PD <sub>50</sub> mg/kg	325 ± 20	
	LD <sub>50</sub> mg/kg	1000 ± 56	
Oral	PD <sub>50</sub> mg/kg		1330 ± 80
	LD <sub>50</sub> mg/kg		2150 ± 148

In frogs (*R. temporaria*) flaccid paralysis and cessation of respiration were obtained after 3 to 10 mg of myanesin injected into the anterior lymph sac. In frogs myanesin also caused a loss of indirect excitability of the muscle (curare-like action) in doses which caused reversible paralysis. In this respect the action of the drug in frogs differed from that in mammals in which loss of indirect excitability of muscle after tolerated doses of myanesin was not observed (16).

The injection of myanesin into the cavity of marine bivalves caused prompt relaxation of the constrictor muscle and opening of the shell (7).

**Acute toxicity.** Death from toxic doses was due to respiratory paralysis. The heart continued beating for a short time after respiration had ceased. Table 1 gives the mean paralysing and mean lethal doses obtained after administration of myanesin by various routes to mice and rats. It shows that there is an adequate margin of safety between paralysing and lethal doses of the drug. The standard safety margin of myanesin calculated according to Foster (43) after intraperitoneal administration to mice was 113 per cent.

Post mortem examinations carried out in animals dying after large doses of myanesin showed moderate engorgement of the liver and spleen, subpleural hemorrhages in the lungs and distention of the right auricle with blood (7).

ble with solutions of sodium chloride, glucose, barbiturates and thiobarbiturates (16, 17)

A solution of 0.01 g of myanesin in 10 drops of concentrated sulphuric acid is slightly red, on addition of a drop of formaldehyde solution a deep red color appears (93)

*The paralyzing action.* Small doses of antodyne or myanesin caused reduction of spontaneous activity and a decrease in muscle tone. Larger doses produced ataxia, flaccid paralysis and loss of the righting reflex. Muscular paralysis was always of the ascending type. The posterior limbs and the lower half of the body were affected first and remained paralyzed longer than the anterior limbs and neck muscles (42, 16). Respiration was not embarrassed even during complete paralysis of skeletal muscles. During the initial phase of the drug action, respiration was sometimes increased in depth and rate. Paralysis was neither preceded nor followed by excitation or convulsions and was followed by complete recovery of muscular power.

Paralysis occurred within 2 minutes after administration of the drug. The depth and duration of paralysis depended on the amount of drug given. In mice after intraperitoneal administration of 250 mg of myanesin per kg, the righting reflex was lost for about 25 minutes. During paralysis the animals remained motionless and did not execute running movement usually observed after administration of small doses of barbiturates or other anesthetics. The animals were completely limp and lacked righting reflexes. They reacted with powerful and sustained muscular contractions to painful stimuli. The pupillary and corneal reflexes and the knee jerk were unchanged. There was no change in the size of the pupils.

During the peak of paralysis after large doses of the drug, the corneal reflex was lost, the pupils were somewhat dilated and reacted sluggishly to light. Nystagmus was observed for short periods of time during the peak of the drug action. There was some dilatation of the pupil in response to nociceptive stimuli but the withdrawal reflex was abolished. Large doses of myanesin caused salivation in rabbits and cats but vomiting was not observed.

Recovery from paralysis was usually rapid. There was some incoordination of movements for about one hour after muscular power was regained. Burke and Linegar (28) observed nausea and vomiting during recovery from paralysis in dogs.

*Route of administration.* Myanesin could be effectively administered to animals by the intravenous, intraperitoneal, intramuscular, subcutaneous, rectal and oral routes. The amount of drug producing paralysis varied greatly with the route of administration (Table 1). On intravenous administration, the effect obtained varied greatly with the speed of injection. In rabbits the largest tolerated dose on rapid injection was about 100 mg per kg whereas as much as 350 mg per kg could be tolerated if the injection was carried out very slowly (16).

*Species sensitivity.* Myanesin produces paralysis in most species of laboratory animals. When the amount of drug required for the production of paralysis was expressed in mg per kg body weight, smaller doses of myanesin were required for the production of paralysis in species of large animals than in species of small



Myanesin injected intravenously to anesthetized rabbits or cats did not cause any alteration of blood pressure or respiration in doses of 10 mg per kg. Larger doses caused a transient fall of blood pressure, bradycardia and a decrease in rate and increase in depth of the respiratory movements. A rise of blood pressure after myanesin was never observed (16). Oostende (99) found in dogs that myanesin in doses which produced relaxation of abdominal muscles (15 mg per kg) did not depress the blood pressure or respiration and did not influence the homeostatic mechanism of blood pressure regulation. Larger doses caused a small and transient fall of blood pressure and slight inhibition of respiration but left the homeostatic mechanisms uninfluenced.

The blood pressure responses to epinephrine, acetylcholine and histamine were not altered even after large doses of myanesin. Myanesin did not influence the depressor effect and slowing of the heart produced by stimulation of the peripheral vagus nerve (7).

*Action on the muscle* Myanesin in concentrations of 1 in 500 produced a slow contracture of the isolated rectus abdominis of the frog (*R. temporaria*). Higher dilutions of the drug were ineffective (9).

Contractures produced in the isolated rectus abdominis muscle of the frog by acetylcholine could not be prevented by myanesin in high dilutions (9).

Myanesin injected intravenously to cats in doses of 10 to 30 mg per kg did not cause any change in the muscle action potential of the tibialis anticus muscle. Doses of 50 mg per kg depressed and 70 mg per kg abolished the muscle action potentials (117).

*The myoneural junction* Intravenous administration of myanesin in doses of 50 mg per kg to chloralosed or decerebrated cats did not influence the indirect excitability of the gastrocnemius muscle. In mice after very large doses of the order of 500 mg per kg there was no response to indirect stimulation but the threshold to direct stimulation was unchanged (9). This effect may have been due to either a depression of neuromuscular transmission or to direct action on the nerve (local anesthetic action). Because indirect excitability was not abolished after systemic administration of large doses of cocaine and procaine, it appears likely that myanesin in very large doses may produce a block at the myoneural junction. This curare-like effect, however, does not play any part in the production of reversible muscular paralysis in mammals because under such conditions paralysis to indirect stimulation is never observed.

*The local anesthetic action* The local anesthetic activity of myanesin was similar to that of procaine when the rabbit's cornea or the motor or sensory nerves of the frog were used as test objects. When examined by the intracutaneous wheal method in guinea pigs, myanesin had only about two-thirds of the activity of procaine (9). As procaine does not produce paralysis on systemic administration, it appears unlikely that the paralyzing action of myanesin would be due to its local action on the peripheral nerves. The opinion that the local anesthetic effect and the paralyzing action are independent properties of the drug is further supported by the observation that the paralyzing drug, benzimidazole, does not possess any local anesthetic action.

*Chronic toxicity* Young growing rats fed for 9 weeks on a diet containing 2 per cent of myanesin did not gain weight as rapidly as the controls which were litter mates. This may have been due to lower food consumption, possibly because of the unpalatability of the drug-containing diet. On post mortem examination, six out of 20 treated rats showed calculi in the bladder. Other organs did not show any macroscopical or microscopical changes (16).

In another chronic toxicity experiment (18), six young Wistar rats weighing 100 to 140 grams received a diet containing 2 per cent of myanesin while six other animals of similar weight and age served as controls. Each animal on the myanesin-containing diet consumed on the average 0.28 gram myanesin per day. The health of all animals was excellent throughout the period of the experiment. During the duration of the experiment, the urine was examined daily for abnormal constituents and the urea contents of the urine of each animal was estimated at frequent intervals.

One rat receiving the myanesin-containing diet passed dark brown urine from the 7th to the 13th and again from the 36th to the 55th day of the experiment. The urine contained protein but gave negative tests for blood and bile pigments. Another myanesin-fed animal passed discolored urine from the 30th to the 34th day. This urine did not contain protein, blood or bile. The urines of the remaining four test animals and of the six control rats did not contain abnormal constituents at any time during the course of the experiment.

The urea output varied greatly from day to day in both test and control animals, but on any one day the values of the controls were always close to those of the test animals. The animals receiving myanesin usually had a somewhat lower urea output and urine volume than the controls but the differences between the control and test values on any one day were smaller than the day-to-day variations of either the test or control animals. On the 78th and 160th day of the experiments, the red and white cells of all rats were counted and the hemoglobin content determined. The counts of the test animals did not differ significantly from those of the controls and were within the normal limits occurring in Wistar rats. On the 55th day of the experiment, three animals were killed, one control rat, one rat which was on the myanesin diet but did not show any symptoms, and the animal (No. 1) which showed dark urine and proteinuria on two occasions. All three animals were in good general health and there were no pathological findings found at post mortem examination. No organs, with the exception of the kidneys, showed histological changes. The only abnormality found was an increase of vacuolation of the glomeruli, which was somewhat more marked in rat No. 1 (which had proteinuria and dark urine) than in the rat which tolerated the myanesin-containing diet without symptoms. Similar abnormalities were present in the kidneys of animals which were on the drug-containing diet for 164 days.

*Effect on the cardiovascular system and respiration* Antodyne in doses of 100 to 400 mg per kg given intravenously to rabbits caused elevation of blood pressure and bradycardia. After larger doses, the rise of blood pressure was later followed by a fall. At the time of onset of paralysis, the blood pressure was usually elevated above the original value (42).

anesthetized cats in which exaggerated knee jerks and tremors were produced by the administration of strychnine, myanesin in small doses abolished the tremor and reduced the knee jerk to its usual size (9) Myanesin also reduced to normal the hyperirritable spinal reflexes produced in monkeys by the injection of neostigmine or strychnine, but did not have any effect on normal reflexes (73)

Stephen and Chandy (117) examined the effect of myanesin on contralateral transmission through the spinal cord When they stimulated one sciatic nerve and recorded the nerve action potentials from the other sciatic nerve, no changes from the normal were observed after injections of 40 mg per kg of myanesin These findings are in disagreement with the results obtained by the reviewer who observed suppression of the crossed extensor reflex in both intact anesthetized and spinal cats after similar and even smaller doses of myanesin

*Effect on facilitatory and inhibitory systems* The loss of postural reflexes, the occurrence of nystagmus, and certain clinical observations in humans show that myanesin possesses a definite action on the basal ganglia and nuclei of the brain stem This action was experimentally investigated by Henneman *et al* (63) Working with cats, they found that the reduction of the knee jerk observed after stimulation of the suppressor centers in the reticular formation could be counteracted by the administration of myanesin On the other hand, the increased knee jerk obtained after stimulation of the facilitatory centers of the reticular formation could be reduced by the administration of myanesin to the size present before stimulation Thus, it appears that myanesin can counteract impulses originating in both the suppressor and excitatory nuclei of the reticular formation These effects were obtained with small doses of myanesin which did not cause paralysis

Inhibition and facilitation of the knee jerk resulting from cortical stimulation were abolished with still smaller doses of myanesin This observation suggests that longer and more complex circuits, such as are usually involved in spasticity, are more vulnerable to myanesin Purely spinal facilitatory and inhibitory reflex arcs examined in decapitate preparations were similarly influenced From this type of evidence, and from electrical studies of segmental spinal reflexes, it appears that myanesin relieves spasticity by reducing tonic extrapyramidal facilitation of stretch reflexes, whatever its source Because facilitation dominates inhibition in spasticity, it is presumably more affected by the drug (63)

*Effects on cortical function* Electroencephalographic studies in humans did not show any evidence of significant alteration in the electrical activity of the cortex after large intravenous doses of myanesin (117, 46) In certain cases which showed increased nervous tension, there was an increase in normal alpha rhythm after myanesin but slow waves were not observed Abnormal waves recorded from the base of the brain disappeared after myanesin (117) Everett and Toman (50) found definite electroencephalographic changes of the sleep type with myanesin but not with benzimidazole when doses were employed which just produced spinal cord effects

Finkelman and Dobin (42b) stimulated the cortex of cats by application of strychnine and recorded potentials from the cortex and the pyramids After

*Effect on the peripheral nerve* Myanesin in doses of 30 mg per kg intravenously did not influence the nerve action potentials, the neuromuscular conduction time, or the nerve conduction velocity in cats. After doses of 50 mg per kg, there was marked depression of the nerve action potentials, and a definite prolongation of the nerve-muscle transmission time and the nerve conduction time (117). The rheobase, chronaxie, galvanic tetanus ratio and repetitive stimuli ratios were unchanged even after the administration of toxic doses (42a).

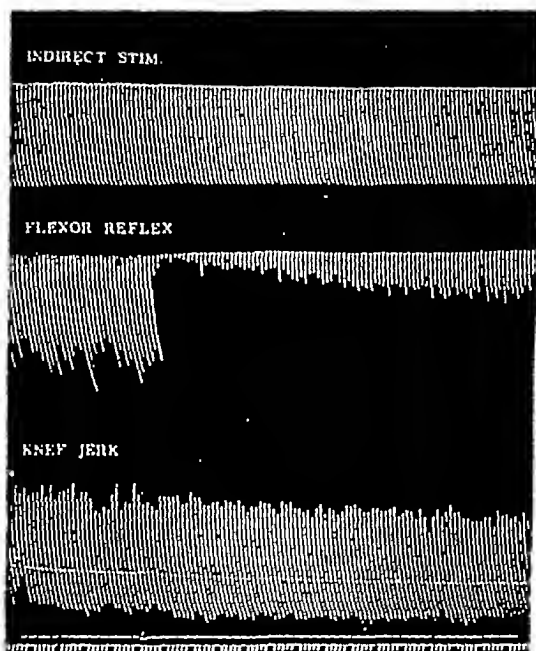


FIG 5 The effect of myanesin on indirect excitability of skeletal muscle, the flexor reflex and the knee jerk. Cat 2.89 kg. Dial anesthesia.

Tracings from above downwards: 1) Stimulation of the carpal flexor muscles of the front leg through its nerve, 2) The flexor reflex, 3) The knee jerk, 4) Signal line and 5) Time in 10 second intervals. At the signal myanesin 40 mg injected intravenously.

*Effect on spinal reflexes* Myanesin had no effect on the normal knee jerk of cats and rabbits in doses which caused muscular relaxation and paralysis. The flexor and crossed extensor reflexes were depressed or abolished after small, non-paralysing doses of the drug. This effect is illustrated in Figure 5 which also shows the lack of curare-like action of myanesin. The depressant action of the drug on multineuron reflexes taken in conjunction with its lack of effect on two-neuron arc reflexes suggests that myanesin possesses a selective depressant action on the interneurons of the spinal cord. An equal degree of depression of the flexor reflex in an anesthetized cat weighing about 3 kg was obtained after intravenous injections of 80 mg benzimidazole, 40 mg myanesin or 25 mg glyketal (7).

Myanesin had a marked effect on exaggerated tendon reflexes. In lightly

anesthetized cats in which exaggerated knee jerks and tremors were produced by the administration of strychnine, myanesin in small doses abolished the tremor and reduced the knee jerk to its usual size (9) Myanesin also reduced to normal the hyperirritable spinal reflexes produced in monkeys by the injection of neostigmine or strychnine, but did not have any effect on normal reflexes (73)

Stephen and Chandy (117) examined the effect of myanesin on contralateral transmission through the spinal cord When they stimulated one sciatic nerve and recorded the nerve action potentials from the other sciatic nerve, no changes from the normal were observed after injections of 40 mg per kg of myanesin These findings are in disagreement with the results obtained by the reviewer who observed suppression of the crossed extensor reflex in both intact anesthetized and spinal cats after similar and even smaller doses of myanesin

*Effect on facilitatory and inhibitory systems* The loss of postural reflexes, the occurrence of nystagmus, and certain clinical observations in humans show that myanesin possesses a definite action on the basal ganglia and nuclei of the brain stem This action was experimentally investigated by Henneman *et al* (63) Working with cats, they found that the reduction of the knee jerk observed after stimulation of the suppressor centers in the reticular formation could be counteracted by the administration of myanesin On the other hand, the increased knee jerk obtained after stimulation of the facilitatory centers of the reticular formation could be reduced by the administration of myanesin to the size present before stimulation Thus, it appears that myanesin can counteract impulses originating in both the suppressor and excitatory nuclei of the reticular formation These effects were obtained with small doses of myanesin which did not cause paralysis

Inhibition and facilitation of the knee jerk resulting from cortical stimulation were abolished with still smaller doses of myanesin This observation suggests that longer and more complex circuits, such as are usually involved in spasticity, are more vulnerable to myanesin Purely spinal facilitatory and inhibitory reflex arcs examined in decapitate preparations were similarly influenced. From this type of evidence, and from electrical studies of segmental spinal reflexes, it appears that myanesin relieves spasticity by reducing tonic extrapyramidal facilitation of stretch reflexes, whatever its source Because facilitation dominates inhibition in spasticity, it is presumably more affected by the drug (63)

*Effects on cortical function* Electroencephalographic studies in humans did not show any evidence of significant alteration in the electrical activity of the cortex after large intravenous doses of myanesin (117, 46) In certain cases which showed increased nervous tension, there was an increase in normal alpha rhythm after myanesin but slow waves were not observed Abnormal waves recorded from the base of the brain disappeared after myanesin (117) Everett and Toman (50) found definite electroencephalographic changes of the sleep type with myanesin but not with benzimidazole when doses were employed which just produced spinal cord effects

Finkelman and Dobin (42b) stimulated the cortex of cats by application of strychnine and recorded potentials from the cortex and the pyramids After

myanesin, 30 mg per kg intravenously, the cortical spikes continued but none could be observed at the pyramid. The synchronous movements of the extremity observed after application of strychnine disappeared after administration of myanesin. From these results it may be concluded that myanesin did not act on cortical cells but impeded transmission through subcortical efferent pathways.

*The anticonvulsant action* Launoy observed in 1910 that guinea pigs treated with antodyne tolerated lethal doses of strychnine without ill effects (78). Myanesin also possessed a powerful antagonistic action to the effects of strychnine. On simultaneous intravenous administration, as little as one-thirtieth of the  $LD_{50}$  of myanesin protected mice from a minimal lethal dose of strychnine. Larger doses of strychnine could be antagonized by proportionally larger doses of myanesin. Myanesin antagonized both the lethal and the convulsant action of strychnine in small, non-paralysant doses and was, in this respect, much more effective than hexobarbital which possessed a protective action only in anesthetic doses (9). Orloff *et al* (100), using timed intravenous infusions of strychnine, found myanesin more effective than trimethadione, phenurone and phenobarbital in elevating the threshold for convulsions and in protecting mice from death.

The antagonistic action of myanesin against metrazol was relatively weak. Large paralyzing doses of myanesin were required to prevent convulsions from metrazol. Smaller doses of myanesin were effective in preventing death but had little effect on the incidence and severity of convulsions (8, 124).

Myanesin effectively antagonized the central convulsant effects of hexachlorocyclohexane in dogs (37).

Antodyne, myanesin and numerous related compounds elevated the threshold to electrically induced convulsions in rats and rabbits. In this respect, antodyne and myanesin were about equally effective as trimethadione (11). Unna *et al* (124) have shown that myanesin prevented death from electroshock in mice and changed the pattern of the seizures. The tonic flexor-extensor phase was abolished and only clonic convulsions were observed. These were of a much more violent nature than in the controls. The effect of myanesin in rabbits undergoing electroshock was studied by Holt *et al* (68b).

*Myanesin antagonists* With the possible exception of strychnine, there is no other drug known at present which has an appreciable analeptic action in myanesin paralysis. The following drugs did not have any appreciable effect on the duration of paralysis in mice: epinephrine, amphetamine, atropine, nikethamide, ephedrine, metrazol, physostigmine, picrotoxin, neostigmine, and strychnine. The duration of paralysis in rabbits was shortened by strychnine if the drug was given intravenously immediately after myanesin was administered (8).

*Synergistic effects* The simultaneous administration of ineffective doses of hexobarbital and myanesin to mice produced narcosis of 30 minutes duration. Small doses of myanesin also increased the depth and duration of hexobarbital anesthesia and suppressed pre-narcotic excitement (16). On joint administration to mice, myanesin and pentobarbital produced an additive effect (128).

The joint action of d-tubocurarine administered with myanesin, as judged by the ability of the animals to maintain themselves on the rotating cylinder, was

less effective than would have been expected on the basis of simple summation of effects. When lethal doses were given, the combined effect of d-tubocurarine and myanesin was potentiated (20).

*Antipyretic and analgesic action.* Antodyne in a large dose had a marked but short-lasting hypothermic action in dogs (47). In guinea pigs made febrile by the injection of sputum of tuberculous patients, antodyne in doses of 100–150 mg per kg caused a marked but transient fall of temperature (42). Myanesin did not possess any analgesic action in rats, mice and rabbits in doses insufficient to cause paralysis (16).

*Effect on urine flow.* Antodyne had a prompt but short-lasting diuretic effect (42). With myanesin, a transient antidiuretic action was observed (18). In rats, doses of 350 mg per kg significantly reduced urine excretion for about 5 hours following administration of the drug but did not greatly influence the total amount of urine excreted within 20 hours.

*Other properties.* Myanesin in dilutions of 1 in 10,000 did not affect the tonus and contractions of the isolated rabbit duodenum. Contraction of the guinea pig ileum caused by histamine or acetylcholine could be partially relaxed by myanesin in a dilution of 1 in 5,000 (16).

Myanesin was unable to protect guinea pigs from dyspnoea and death produced by histamine or acetylcholine aerosols (7).

It was not possible to sensitize guinea pigs to antodyne or myanesin (79a, 7). The repeated cutaneous application of 10 ml per kg of a 5 per cent aqueous solution of antodyne discolored the skin of rats but did not cause irritation (79a). An exposure to antodyne mist for one hour produced slight temporary respiratory irritation in hamsters (79a).

Injected intravenously in rabbits, myanesin in doses of 110 mg per kg given on two successive days did not affect the blood sugar and blood urea levels. This dosage was also without effect on the number of red and white cells. The hemoglobin content of the blood was slightly lowered after injection of the drug but this decrease was not significant. There was also a slight relative neutropenia and lymphocytosis after administration of large doses of myanesin to rabbits (7).

Myanesin in large paralyzing doses did not depress the tail response in morphinized mice (101). This reflex is regularly depressed after small doses of d-tubocurarine or  $\beta$ -erythroidine.

A marked delay in the onset of rigor mortis could be obtained in rabbits by the administration of 300 mg per kg of myanesin 30 minutes before the animal was sacrificed (4).

*The antibacterial action.* Myanesin possessed a bactericidal action on various gram-positive and gram-negative bacteria in concentrations from 0.2 to 1 per cent. The effective concentrations against *Streptococcus hemolyticus*, *Pseudomonas pyocyanea* and *Clostridium Welchii* were 0.2, 0.8 and 0.4 per cent, respectively. The bactericidal action of myanesin was not greatly inhibited in the presence of 10 per cent horse blood. Tests for inhibition of phagocytosis, carried out by the technique of Welch and Hunter (129) using artificially opsonized staphylococci,

showed that myanesin was significantly less toxic to leucocytes than was phenol in concentrations of 0.2 and 0.4 per cent. In higher concentrations, the toxicity of the two compounds was similar. Myanesin did not exert any chemotherapeutic activity in mice infected with hemolytic streptococci or *Ps. pyocyanea* (7).

*Method of determination.* Three methods for the determination of myanesin in blood and body fluids have been described. The method of Wyngaarden *et al.* (136) depends upon nitration of myanesin in aqueous solution and the development of a strong yellow-green color on addition of sodium hydroxide. Titus *et al.* (118) developed two methods of determination: one involves coupling of myanesin with diazotized 2,4-dinitroaniline, and the other a colorimetric determination of the formaldehyde resulting from periodate oxidation. The coupling method is most sensitive and permits the determination with accuracy of 2 micrograms per ml. The smallest amount of myanesin which can be measured by the other two methods is about 5 micrograms per ml.

Morch (93) described 2 methods suitable for the quantitative determination of myanesin in pharmaceutical preparation.

*Metabolism of myanesin.* Myanesin is quickly metabolized to a physiologically inactive compound in the body. Suitable extracts of the urine collected from rabbits or rats after large doses of myanesin did not cause paralysis in mice (16, 18). Chemical analysis showed that, in dogs, only 0.1 to 2 per cent of the administered dose was excreted as free myanesin (136, 118), while 32 to 42 per cent were excreted in conjugated form (136).

Further studies disclosed the presence of at least two different metabolic products of myanesin in the urine (54, 105). The metabolite excreted in large quantities was identified as  $\beta$ -(*o*-toloxyl)-lactic acid. This compound melted at 146°C, was optically inactive, and had a neutral equivalent of 198. Because of its low toxicity, it appeared to be a true detoxification product. It was pharmacologically inert and did not possess any paralyzing myanesin-like action in doses as high as 1200 mg per kg (105).

The other metabolic compound appearing in the urine after administration of myanesin gave a positive test with Ehrlich's diazo reaction (18). The diazo reaction observed conformed to the type B reaction of Hunter (69). The chemical structure of this compound has not yet been determined but it appears probable that it represents a further step in the degradation of myanesin. Although this metabolite was excreted in small quantities, it was present in the urine as early as 15 minutes after an oral dose of myanesin (21). The reaction was given by urine passed up to 8 hours after a single dose of myanesin. Rats fed for 2 months a diet containing 2 per cent of myanesin continued to excrete the metabolite for 2 days after myanesin has been discontinued. The intensity of the color in the urine was proportional to the amount of drug taken.

*Phenolic constituents in urine.* The excretion of phenols in urine after large doses of antodyne was unchanged (42). The phenolic constituents in the urine of rats which had been fed a diet containing 2 per cent of myanesin for 150 days were estimated by the method of Volterra (127). There was a small increase in free volatile phenols and a very marked increase of the conjugated phenols. The amount of



aromatic hydroxy acids and residual phenols was about twice as large in the test animals as in the controls. An aqueous solution of myanesin subjected to analysis by Volterra's method was quantitatively indicated in the residual phenol fraction (18).

The nature of the conjugated phenols excreted after the administration of myanesin has been further investigated. It was found that 59 per cent of the total conjugated phenols or 3.7 per cent of the total myanesin ingested was conjugated to glucuronic acid, and 14.4 per cent of the conjugated phenols or 0.13 per cent of the ingested dose of myanesin was excreted as a sulphuric acid conjugate (18).

*Plasma levels* Wyngaarden *et al* (136) correlated the plasma levels of myanesin with the effects observed in dogs. After intravenous administration of 50 mg per kg of myanesin, flaccid paralysis was observed with blood levels of about 5 mg per cent. Paralysis was very transient, but unsteadiness and muscular weakness persisted for about 20 minutes. During this time, the plasma levels were 2.4 mg per cent. The myanesin concentration dropped to about 0.8 mg per cent 40 minutes after administration of the drug. No free myanesin could be detected in the 90-minute plasma sample of any of the dogs.

*Rate of disposition* The rate at which myanesin was detoxified in rabbits was determined. Myanesin was injected intravenously as a 10 per cent solution at a rate of 100 mg per minute. The mean lethal dose was 220 mg per kg. To obtain an indication of the rate of detoxification, it was assumed that death of the animal would occur when an amount of myanesin equivalent to the  $LD_{50}$  would be present in the body in unchanged form. The occurrence of death after injection of one half of the  $LD_{50}$  repeated at various intervals was therefore observed. The difference between the  $LD_{50}$  given in one dose and the lethal dose after fractional doses was taken to be equal to the amount of drug detoxified during the period of time elapsing between the administration of the first fractional dose and the death of the animal. When doses were given at 10-minute intervals, on the average 40 per cent of the  $LD_{50}$  (*i.e.*, 88 mg per kg) was detoxified in 21 minutes. Therefore, 4.2 mg per kg were detoxified each minute. The corresponding values obtained when myanesin was given at intervals of 15 and 20 minutes were 3.7 and 3.9 mg per kg per minute, respectively. Thus it appears that rabbits can dispose of about 4 mg myanesin per kg each minute (7). Cats tolerated the intravenous infusion of 4.5 mg of myanesin per minute per kg body weight for 3 or 4 hours (16).

Morrison *et al* (95) examined the rate of disposal in dogs by a more direct method. They injected a priming dose of 60 mg per kg of myanesin intravenously and 5 minutes later started a continuous infusion of myanesin at a rate of 1 ml per minute. The concentration of the drug was adjusted so as to give each animal either 1 or 2 mg of myanesin per kg per minute. Blood samples for determination of plasma concentrations were taken at 30-minute intervals for 2 hours during the period of infusion and at hourly intervals thereafter. Infusion of 2 mg per kg per minute resulted in increasing plasma concentrations and 1 mg per kg was insufficient to maintain plasma levels.

*Distribution in body fluids and tissues* Myanesin is widely distributed in the

body approximately according to the water content of each tissue. A notable exception to this was the brain in which the ratio of tissue to plasma was always more than unity and averaged about 2. The concentrations of myanesin in spinal fluid and saliva were consistently lower than in plasma (94, 95, 103). Data on the concentration of myanesin in the spinal cord have not been published.

*Related compounds* Most ethers of glycerol of the structure  $R-O-CH_2-CHOH-CH_2OH$  produced transient paralysis qualitatively similar to that observed after antodyne or myanesin (24, 11, 80).

When R was an aliphatic radical, straight chain alkyls contributed more to the paralyzing activity than branched chain isomers or unsaturated radicals. The *n*-amyl ether was the most potent compound of the aliphatic series. It was about as active as antodyne and about one-third as active as myanesin.

The activity of compounds in which R was a substituted benzene nucleus varied with the position and kind of the substituent group. Compounds with a small alkyl or alkoxy group or chlorine in the ortho position possessed strongest paralyzing action. Compounds with these radicals in meta or para position were less active than the ortho isomers. The presence of a hydroxy, amino, amido, ester or hydroxy-alkyl group or multiple substitution in the benzene ring with alkyls, halogens or both decreased paralyzing activity (11).

Compounds with lower solubility than myanesin showed, for the most part, a slower onset and longer duration of action. The rather insoluble 3-(1'-methyl, 4'-isopropyl) propane-1,2-diol on oral administration to dogs produced more prolonged and constant blood levels than myanesin (29).

Stereochemical configuration did not appear to influence paralyzing activity as judged by the similar activity of the levo and racemic guaiacol glycerol ethers (112).

The effect of substitutions in the glyceryl side-chain depended on the structure and position of the substituent group. Methyl substitution on the  $C_2$  atom of the glyceryl side-chain did not materially alter paralyzing activity but substitution on the  $C_1$  atom decreased activity. Substitution in the hydroxyl groups decreased or destroyed paralyzing activity.

An increase or decrease in the length of the glycerol chain caused a decrease or disappearance of paralyzing properties as witnessed by the slight activity or inactivity of the glycol, erythritol and mannitol homologues (11).

Alpha substituted glycidyl ethers were generally more potent than the corresponding glycerol derivatives. Replacement of the hydroxy groups of  $C_1$  and  $C_2$  atoms by aliphatic groups decreased the potency (67).

The alpha thioethers and sulphones of glycerol also had paralyzing activity but were more toxic than the oxygen ethers.

The 3-(2'-methoxyphenoxy)propane-1,2-diol which possesses paralyzing properties of a similar order as myanesin has been sold for many years as an expectorant under the proprietary name of Resyl.

Myanesin acid succinate possessed pharmacological properties qualitatively similar to myanesin. It was less toxic and had a much weaker paralyzing action. In doses not causing paralysis, it had a longer duration of action than myanesin.

Liver homogenates hydrolysed myanesin acid succinate into myanesin and succinic acid and the compound was dealt with in a similar way *in vivo* (106, 19)

Certain 2-substituted-1, 3-propanediols had a stronger anticonvulsant action and a weaker paralysing action than myanesin and similar compounds 2,2-Diethyl-1, 3-propanediol, called DEP, the outstanding compound of the series was as active as phenobarbital in preventing convulsions and deaths from lethal doses of metrazol in mice. It was more effective than phenobarbital or myanesin in antagonizing the convulsant and lethal effects of strychnine. Suitable doses of DEP also prevented or modified electroshock seizures in mice and rabbits (14a)

### *The Clinical Use of Myanesin*

*Effect of administration in humans* The slow intravenous administration of 1 gram of myanesin to adults did not cause any effects (83). A similar dose given more rapidly caused a subjective sense of warmth, relaxation and slight giddiness but no impairment of mental faculties. There was diminution of muscle tone without interference with voluntary muscle control. Strength measured on a dynamometer was not altered. Coarse nystagmus in all directions, loss of eye convergence and slurred speech were also observed. Rarely patients became faint when placed upright while under drug action. During or after administration, no apprehension was felt (83, 117, 46, 110)

After administration of somewhat larger doses (2 grams or 30 mg per kg), most patients complained of feeling "dopey" or relaxed. After completion of the injection, the depression disappeared within 2 to 3 minutes but the relaxed feeling persisted for about an hour (117). Baisi (2a) observed vomiting of central origin (which was not preceded by nausea) after parenteral administration of myanesin.

The oral administration of myanesin in 1 gram doses, as a rule, did not cause any symptoms provided that it was given after meals. A similar dose given on an empty stomach sometimes caused transient giddiness and a feeling of relaxation. A few patients experienced "heart burn" and nausea (21). Certain patients exhibited a mild degree of euphoria for 1 to 2 hours following administration of the drug (117, 62, 7)

*Use during anesthesia* For the performance of numerous surgical operations, adequate muscular relaxation is required. To obviate the risk concomitant to deep anesthesia, the use of curare with light anesthesia has been advocated (57). Curare used in this way produces paresis or paralysis of the skeletal muscles by blocking neuromuscular transmission. Myanesin is used in order to depress the reflex hyperexcitability present during light anesthesia. It is not used clinically for its paralytic action which requires amounts greater than those usually given to patients (10)

The effect of myanesin in anesthesia was described for the first time by Mallinson (83). He used the drug in 112 cases in conjunction with pentothal and nitrous oxide or cyclopropane and obtained excellent relaxation with doses ranging from 0.5 to 2 grams intravenously (about 7 to 28 mg per kg). Respiration and circulation were not impaired and no complications attributable to the drug were

body approximately according to the water content of each tissue. A notable exception to this was the brain in which the ratio of tissue to plasma was always more than unity and averaged about 2. The concentrations of myanesin in spinal fluid and saliva were consistently lower than in plasma (94, 95, 103). Data on the concentration of myanesin in the spinal cord have not been published.

*Related compounds* Most ethers of glycerol of the structure  $R-O-CH_2-CH(OH)-CH_2OH$  produced transient paralysis qualitatively similar to that observed after antodyne or myanesin (24, 11, 80).

When R was an aliphatic radical, straight chain alkyls contributed more to the paralyzing activity than branched chain isomers or unsaturated radicals. The *n*-amyl ether was the most potent compound of the aliphatic series. It was about as active as antodyne and about one-third as active as myanesin.

The activity of compounds in which R was a substituted benzene nucleus varied with the position and kind of the substituent group. Compounds with a small alkyl or alkoxy group or chlorine in the ortho position possessed strongest paralyzing action. Compounds with these radicals in meta or para position were less active than the ortho isomers. The presence of a hydroxy, amino, amido, ester or hydroxy-alkyl group or multiple substitution in the benzene ring with alkyls, halogens or both decreased paralyzing activity (11).

Compounds with lower solubility than myanesin showed, for the most part, a slower onset and longer duration of action. The rather insoluble 3-(1'-methyl, 4'-isopropyl) propane-1,2-diol on oral administration to dogs produced more prolonged and constant blood levels than myanesin (29).

Stereochemical configuration did not appear to influence paralyzing activity as judged by the similar activity of the *levo* and racemic guaiacol glycerol ethers (112).

The effect of substitutions in the glyceryl side-chain depended on the structure and position of the substituent group. Methyl substitution on the  $C_2$  atom of the glyceryl side-chain did not materially alter paralyzing activity but substitution on the  $C_1$  atom decreased activity. Substitution in the hydroxyl groups decreased or destroyed paralyzing activity.

An increase or decrease in the length of the glycerol chain caused a decrease or disappearance of paralyzing properties as witnessed by the slight activity or inactivity of the glycol, erythritol and mannitol homologues (11).

Alpha substituted glycidyl ethers were generally more potent than the corresponding glycerol derivatives. Replacement of the hydroxy groups of  $C_1$  and  $C_2$  atoms by aliphatic groups decreased the potency (67).

The alpha thioethers and sulphones of glycerol also had paralyzing activity but were more toxic than the oxygen ethers.

The 3-(2'-methoxyphenoxy)propane-1,2-diol which possesses paralyzing properties of a similar order as myanesin has been sold for many years as an expectorant under the proprietary name of Resyl.

Myanesin acid succinate possessed pharmacological properties qualitatively similar to myanesin. It was less toxic and had a much weaker paralyzing action. In doses not causing paralysis, it had a longer duration of action than myanesin.

Liver homogenates hydrolysed myanesin acid succinate into myanesin and succinic acid and the compound was dealt with in a similar way *in vivo* (106, 19)

Certain 2-substituted-1, 3-propanediols had a stronger anticonvulsant action and a weaker paralyzing action than myanesin and similar compounds 2,2-Diethyl-1, 3-propanediol, called DEP, the outstanding compound of the series was as active as phenobarbital in preventing convulsions and deaths from lethal doses of metrazol in mice. It was more effective than phenobarbital or myanesin in antagonizing the convulsant and lethal effects of strychnine. Suitable doses of DEP also prevented or modified electroshock seizures in mice and rabbits (14a)

### *The Clinical Use of Myanesin*

*Effect of administration in humans* The slow intravenous administration of 1 gram of myanesin to adults did not cause any effects (83). A similar dose given more rapidly caused a subjective sense of warmth, relaxation and slight giddiness but no impairment of mental faculties. There was diminution of muscle tone without interference with voluntary muscle control. Strength measured on a dynamometer was not altered. Coarse nystagmus in all directions, loss of eye convergence and slurred speech were also observed. Rarely patients became faint when placed upright while under drug action. During or after administration, no apprehension was felt (83, 117, 46, 110).

After administration of somewhat larger doses (2 grams or 30 mg per kg), most patients complained of feeling "dopey" or relaxed. After completion of the injection, the depression disappeared within 2 to 3 minutes but the relaxed feeling persisted for about an hour (117). Baisi (2a) observed vomiting of central origin (which was not preceded by nausea) after parenteral administration of myanesin.

The oral administration of myanesin in 1 gram doses, as a rule, did not cause any symptoms provided that it was given after meals. A similar dose given on an empty stomach sometimes caused transient giddiness and a feeling of relaxation. A few patients experienced "heart burn" and nausea (21). Certain patients exhibited a mild degree of euphoria for 1 to 2 hours following administration of the drug (117, 62, 7).

*Use during anesthesia* For the performance of numerous surgical operations, adequate muscular relaxation is required. To obviate the risk concomitant to deep anesthesia, the use of curare with light anesthesia has been advocated (57). Curare used in this way produces paresis or paralysis of the skeletal muscles by blocking neuromuscular transmission. Myanesin is used in order to depress the reflex hyperexcitability present during light anesthesia. It is not used clinically for its paralytic action which requires amounts greater than those usually given to patients (10).

The effect of myanesin in anesthesia was described for the first time by Mallinson (83). He used the drug in 112 cases in conjunction with pentothal and nitrous oxide or cyclopropane and obtained excellent relaxation with doses ranging from 0.5 to 2 grams intravenously (about 7 to 28 mg per kg). Respiration and circulation were not impaired and no complications attributable to the drug were

encountered. The tone of the musculature of the intestinal tract was unchanged or slightly diminished (85, 90). The postoperative condition of patients given myanesin was better than that of patients receiving spinal or deep general anesthesia or curare. Mallinson stated that myanesin had advantages over curare because of the wider margin of safety, adequate abdominal relaxation without intercostal paralysis, the potentiation of barbiturate action and the absence of bronchospasm and salivation. The shock-like state which is sometimes observed after the administration of curare has not been noted after myanesin (84). These results were confirmed by Turnbull (123), Bistrom and Vukari (22) and Musgrove (96).

Lyall (82) obtained satisfactory results with myanesin in robust individuals who did not relax easily with ether. In very ill patients, and in thoracic surgery where controlled respiration is of advantage, curare seemed preferable. Ballantine (3), Marshall (89) and Macar (82a) also commended myanesin for producing satisfactory muscular relaxation without respiratory depression. Because of the occurrence of venous thrombosis after administration of a 10 per cent solution, they recommended the use of more dilute solutions which did not cause ill effects. Marston (90) and Carman (31) were impressed by the small amount of myanesin required to secure relaxation in many abdominal operations. Wilson and Gordon (134) and Davison (38) recommended myanesin as an aid to anesthesia in children.

Mallinson (86), in summarizing his experience of 1500 myanesin injections, stated that the principal field for myanesin was abdominal surgery, hemorrhoidectomy and colpopерineal repair. Used jointly with pentothal, myanesin was of little value in facilitating intubation. It abolished laryngeal spasm when used during deeper levels of anesthesia. Myanesin was also useful in tonsillectomy and Caesarean section, when gas and oxygen and pentothal were used as the anesthetic agents. Mallinson believes that relaxation with myanesin is as good as with curare. This view was contradicted by Gray (55) and Woolmer (135) who found myanesin ineffective in reasonable dosage. Dinnick (39) abandoned the use of the drug because the relaxant effect was not as pronounced as that produced by curare. Dale (35, 36) found the action of myanesin unpredictable. In some cases, relaxation was comparable to that associated with spinal analgesia, while in others relaxation was absent or poor. Because relaxation of skeletal and abdominal muscles in the absence of stimulation of the peritoneum was good, he used myanesin successfully in gynecology for examinations under anesthesia. Myanesin was also of value in orthopedic operations for manipulation of the spine (36).

#### *The Use of Myanesin in Nervous Disease*

The effects of myanesin on experimental animals suggested that the drug may be of value in certain nervous disease where spasticity, tremor and involuntary movements were a factor.

*Paralysis agitans.* Stephen and Chandy (117) were the first to show that the intravenous injection of myanesin in about 1 gram doses abolished the tremor

and rigidity of patients suffering from paralysis agitans. The symptoms remained in abeyance for 30 to 60 minutes and were less severe than usual for about 12 hours. Voluntary muscular power was not impaired. These results were confirmed and amplified by Schlesinger *et al* (110) who thought that patients with rigidity have an unusual sensitivity to myanesin. Hunter and Waterfall (71) and others (70, 87, 46) also found myanesin effective in paralysis agitans on parenteral administration. Hay (62), treating paralysis agitans in psychotic patients, gained the impression that the remission of symptoms lasted the longer, the more recent the onset of the Parkinson syndrome.

Berger and Schwartz (21) found that myanesin administered orally was effective in certain cases of paralysis agitans. Although the effect of the drug on oral administration was not as spectacular as that observed after parenteral administration, it was of longer duration and did not cause side effects. Berger (7) treated 47 cases of paralysis agitans of various etiology with myanesin orally. Some decrease of rigidity and tremor was observed in 22 cases, 21 patients were unchanged and in four the tremor became temporarily worse. Cases of post-encephalitic, idiopathic and arteriosclerotic origin appeared to respond equally well. The oral efficacy of myanesin was recently confirmed by Gammon and Churchill (46).

Myanesin is said to have an unquestionably greater effect in cases of Parkinsonian rigidity and tremor than any other therapy short of narcosis (46). These results are of particular interest in view of the fact that myanesin differs from most other drugs recommended for the treatment of paralysis agitans in not possessing atropine-like action (14).

*Involuntary movements* The efficacy of myanesin in suppressing or diminishing athetotic and chorea-like movements has repeatedly been shown (117, 110, 21, 46, 108). Athetoid movements in cerebral palsy sufferers, and those observed in bilateral athetosis and dystonia musculorum deformans, responded equally well. Senile tremor was also greatly improved but cases of Huntington's and congenital chorea were unaltered (46). Gammon and Churchill (46) observed an increase of the intention tremor in 2 cases of multiple sclerosis but the reviewer noted a favorable effect on intention tremor and head wagging in three out of 19 cases of multiple sclerosis. Myanesin was also found of considerable value for the amelioration of the jerking movements of the head observed in spasmodic torticollis (7). It proved of no value in four cases of hereditary intention tremor occurring without other neurological symptoms.

*Spasticity* The status of myanesin in spasticity due to upper motor neuron lesion is not yet clearly defined. After intravenous administration of the drug, Stephen and Chandy (117) observed complete flaccidity in a congenital spastic child but did not note any change in the degree of spasticity in a case of spastic paraplegia due to a tuberculous lesion involving the thoracic vertebrae. Schlesinger *et al* (110) have shown that myanesin in suitable dosage has a pronounced though evanescent effect upon the isolated or partly isolated spinal cord. Mather (91) reported immediate and pronounced relaxation of the spastic muscles of the lower limbs in cases of spastic hemiplegia, paraplegia, disseminated sclerosis and other upper motor neurons lesions after an intravenous injection of 1 gram of

myanesin. The effect lasted for 5 or 6 hours. After oral administration of myanesin to certain hemiplegic patients, a decrease of spasticity and recovery of some of the voluntary movements were observed (21). The effect of the drug manifested itself mainly by improvements of the physical performance of the patients. There was little or no change in the tendon reflexes and clonus.

*Muscle spasm.* Schlesinger *et al* (109, 110) believe that acute muscle spasm constitutes the major indication for the use of the drug. Myanesin was particularly effective for the relief of muscle spasm due to acute low back and cervical spine syndromes. The drug also proved of value as an aid or substitute for traction (109, 36), for the reduction of major fractures in muscular males (36), for the evaluation of the reversibility of contractures and deformities, and for facilitation of physical therapy (110). Organic facial hemispasm or fasciculations in amyotrophic lateral sclerosis did not show any alteration after myanesin (46).

*Tetanus.* All the symptoms of experimental tetanus in mice could be abolished by myanesin (9). Belfrage (6) used myanesin intramuscularly in 2 cases of tetanus and found it more effective than curare. The value of myanesin in tetanus has been confirmed by others (89, 109, 46, 77, 38a). Torrens *et al* (122) reported a carefully studied severe case of tetanus in which myanesin gave much benefit. They found that the intravenous injection of 1 gram of the drug in a 5% solution abolished the spasms and enabled the patient to take nourishment. The reflexes remained brisk and clonus persisted. They advocate further clinical trials by the oral, intramuscular and intravenous routes.

*Convulsive states.* Hunter and Waterfall (71) tried the effect of myanesin in 3 cases of continuous epileptic attacks and observed immediate disappearance of the seizures after intravenous doses of 0.4 to 1.0 gram. Schlesinger (109) also found myanesin of value in status epilepticus. Gammon and Churchill (46) observed the disappearance of the characteristic spike and dome waves pattern in the electroencephalograms of six cases of true petit mal. Convulsive cases with focal lesions and two cases of petit mal associated with generalized seizures were unaffected.

Judged by animal experiments, myanesin should be the drug of choice in strychnine poisoning. The use of the drug for this purpose has not been reported. Myanesin was not of much value for the prevention of traumatic accidents during convulsive shock therapy (109). Doses of 30 mg per kg myanesin did not diminish the intensity or the duration of therapeutic electroshock convulsions (62) but shortened the period of apnea following the convulsions (Unna, personal communication). Holt *et al* (68a) found that dilantin markedly enhanced the ability of myanesin to soften convulsive rigor.

*Psychoses.* Schlesinger (110) used myanesin in two cases of catatonia without benefit. Gammon and Churchill (46) observed improvement in a patient with reactive depression. A deteriorated negativistic schizophrenic became communicative for the first time in years and an agitated patient became calm and communicative under the action of the drug. Hay (62) observed pleasant relaxation or euphoria without other changes in the mental condition of four schizophrenics. Three deteriorated catatonics did not show any psychic effects and remained



mute The changes in manic-depressive patients were minimal and of short duration

Schlan and Unna (110b) found in patients with anxiety states that myanesin allayed anxiety without clouding consciousness The drug also had a sedative action in manic depressive psychoses In schizophrenic patients a sedative action was noted only when the environment was not disturbing

*Miscellaneous conditions* Myanesin was of no value in two cases of Friedreich's ataxia (7) In the treatment of various arthritic symptoms (21, 109), myanesin was sometimes helpful but it is not clear whether the effect of the drug in these conditions was due to its muscle relaxing effect, its antipyretic or analgesic action, or to other factors A case of true gouty arthritis responded dramatically to the administration of the drug by mouth

In patients addicted to morphine and heroine, myanesin abolished scmatic symptoms of withdrawal such as yawning, nausea, vomiting and leg cramps but did not affect craving for the drug (110b) Myanesin also promptly abolished the tremor and anxiety of chronic alcoholics in abstinence (110b)

Stephen and Chandy have shown that myanesin is effective in suppressing intractable pain of thalamic origin (117) In tabes, spontaneous pain was abolished and the exaggerated second-pain in response to pinprick was reduced without loss of pinprick perception Causalgic pain was relieved for a short time and improved for 24 hours Phantom limb pain was unaffected (46) Brooks *et al* (26) found that the painful limitations of movements in two cases of polyomyelitis were unaffected

### *Toxic Effects and Complications*

*Hemolysis and hemoglobinuria* Pugh and Enderby (102) have shown that myanesin had hemolytic properties *in vitro* in concentration of 1 in 200 The hemolytic action of the drug was increased by the solvents used in the preparation of the commercially available solutions (64) The solvents used were ethanol and propylene glycol (56) Ogilvie *et al* (98) showed that a 10 per cent commercial solution of myanesin caused flocculation of blood

When myanesin solutions were injected into a vein at the wrist and blood samples withdrawn from a vein in the antecubital fossa were examined for the presence of hemolysis, the injection of 1.5 cc of a 5 per cent solution caused considerable lysis but a similar amount of a 1 per cent solution did not cause any change from the control In these experiments, a commercially prepared solution of myanesin was used and no attempt was made to determine whether myanesin or the solvents were responsible for hemolysis (102) The occurrence of intravascular hemolysis without hemoglobinuria after intravenous myanesin has been observed by Lyall (82) and Wilson and Gordon (134) Pugh and Enderby (102) observed hemoglobinuria following the intravenous use of myanesin in three cases Samples of urine voided soon after the injection were discolored and contained hemoglobin, but no hemoglobin was found in subsequent samples over a period of 2 days Stephen and Chandy (117), Noble (97), Brooks *et al* (26) and Marshall (89) noticed intense hemoglobinuria after administration of myanesin

in 10 per cent solution by the intravenous route Pugh and Enderby believe that intravenous hemolysis occurs with every injection of strong solutions of myanesin Because of the high threshold value of hemoglobin, hemoglobinuria is seen only occasionally They consider preparations of myanesin, as constituted at present, unsatisfactory for intravenous use because of the danger of blockade of the kidney tubules with acid hematin crystals formed from the hemoglobin in acid urine Wilson (133), Hay (62) and Enderby (41) felt that the solvent and not myanesin itself may be responsible for hemolysis

Administration of more dilute solutions (5 per cent or less) did not cause hemoglobinuria or other untoward effects in large series of cases (110, 109, 46, 122, 89, 31, 96) According to Mallinson (86), fewer than 20 cases of hemoglobinuria have reported out of an estimated 10,000 administrations of myanesin He believes that, in certain patients, the discolored urine may be due to an abnormal pigment and not to hemoglobin Mallinson (86) and Wilson and Gordon (134) presented experimental evidence to show that myanesin increased the fragility of the red cells, but Torrens *et al* (122) found a normal red cell fragility curve after 12 grams of myanesin had been given as a 5 per cent solution in divided doses After oral administration of myanesin, hemoglobinuria has not been observed (41, 21)

*Kidney damage and anuria* Hewer and Woolmer (65, 66), Dinnick (39) and Goodier and Goodhart (48) described cases of fatal anuria after the intravenous use of myanesin during anesthesia The necropsy findings were similar to those observed after incompatible blood transfusions and may have been due to intravascular hemolysis followed by deposition of pigment in the renal tubules The histological picture of the kidneys also suggested that myanesin may cause cortical ischemia Mallinson (86) reported uremia in two patients who had myanesin during anesthesia He believes that the uremia in these cases and in the case of Hewer and Woolmer was produced by the trauma of the operation and that it belongs to the "crush syndrome without crush injury" picture (85) He described the occurrence of fatal anuria of a similar type in a case anesthetized with thiopental and curare (88)

*Venous thrombosis* Opinion varies as to the cause and frequency of this complication Mallinson (84, 86) and Vartan (125) reported complete freedom from this complication and drew attention to the possibility of pentothal being the cause Stephen and Chandy (117) recorded seven cases of localized thrombophlebitis in a series of 50 administrations All patients recovered within 48 hours Griffith and Cullen (56) had four cases of thrombophlebitis among 120 patients and Musgrove (96) observed this complication in 10 out of his 200 patients Several authors reported single cases of venous thrombosis (126, 55, 134, 39)

*Intra-arterial injection* Ogilvie *et al* (98) described a case of gangrene of the hand and forearm after accidental injection of myanesin Brooks *et al* (26) observed hemolysis in the brachial vein after an injection of the drug into the brachial artery

*Circulatory effects* Hunter (70) saw a simultaneous failure of circulation and respiration of central origin after intravenous injection of 3 grams of myanesin This was at once reversed by 5 cc of nikethamide intravenously Lyall (82)

observed falls of the systolic and diastolic blood pressure varying as a rule between 10 and 25 mm. In elderly individuals, the falls were greater but never caused anxiety. Cowen (34) observed a partial heart-block shortly after administration of myanesin.

#### GLYKETAL AND OTHER 2,2-ALKYL-4-HYDROXYMETHYL-1,3-DIOXOLANES

It has recently been shown that certain 2-substituted-4-hydroxymethyl-1,3-dioxolanes caused effects on the central nervous system similar to those caused by alpha substituted ethers of glycerol (15, 12). Fifty compounds of this type have been examined and it was found that 2-methyl,2-*n*-amyl-4-hydroxymethyl-1,3-dioxolane, named glyketal, possessed the strongest paralysing activity. The pharmacological properties of glyketal have been investigated (13).

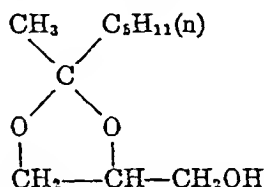


Fig. 6 Structural formula of glyketal

**Physical and chemical properties** Glyketal is a colorless, viscous liquid with a faint fruity odor, it boils at 128–132°C at 10 mm pressure. Glyketal hydrolyses slowly on standing. It is only very slightly soluble in water but forms fairly stable emulsions.

**Effect on animals** The action of glyketal in laboratory animals was very similar to that of myanesin. It caused muscular relaxation after small doses and paralysis with a loss of the righting reflex after larger doses. The drug was a somewhat more effective paralysing agent than myanesin and possessed a similar toxicity.

After intravenous administration of the compound to rabbits, the corneal reflex was lost during the peak of the drug action. During this time, some animals also showed nystagmus. Lethal doses caused death by respiratory paralysis. Paralysis was not preceded or followed by excitation. The lower segments of the spinal cord appeared to be affected first by the drug and were the last to recover from its effects.

**Effect on circulation and respiration** In the intact animal, respiration during paralysis was not affected or was somewhat increased in rate and depth. In cats anesthetized with dial, intravenous injections of glyketal in doses of 5 mg per kg did not affect respiration but produced a slight and transient fall of blood pressure and a decrease in heart rate. Larger doses had a greater depressor effect. Atropine affected neither the fall of blood pressure nor the bradycardia produced by the drug.

**Effect on the nervous system** Glyketal did not possess any action on the peripheral nerves. It did not influence neuromuscular transmission and did not have any curare-like action. It also did not affect two neuron arc reflexes such as the

in 10 per cent solution by the intravenous route Pugh and Enderby believe that intravenous hemolysis occurs with every injection of strong solutions of myanesin Because of the high threshold value of hemoglobin, hemoglobinuria is seen only occasionally They consider preparations of myanesin, as constituted at present, unsatisfactory for intravenous use because of the danger of blockade of the kidney tubules with acid hematin crystals formed from the hemoglobin in acid urine Wilson (133), Hay (62) and Enderby (41) felt that the solvent and not myanesin itself may be responsible for hemolysis

Administration of more dilute solutions (5 per cent or less) did not cause hemoglobinuria or other untoward effects in large series of cases (110, 109, 48, 122, 89, 31, 96) According to Mallinson (86), fewer than 20 cases of hemoglobinuria have reported out of an estimated 10,000 administrations of myanesin He believes that, in certain patients, the discolored urine may be due to an abnormal pigment and not to hemoglobin Mallinson (86) and Wilson and Gordon (134) presented experimental evidence to show that myanesin increased the fragility of the red cells, but Torrens *et al* (122) found a normal red cell fragility curve after 12 grams of myanesin had been given as a 5 per cent solution in divided doses After oral administration of myanesin, hemoglobinuria has not been observed (41, 21)

*Kidney damage and anuria* Hewer and Woolmer (65, 66), Dinnick (39) and Goodier and Goodhart (48) described cases of fatal anuria after the intravenous use of myanesin during anesthesia The necropsy findings were similar to those observed after incompatible blood transfusions and may have been due to intravascular hemolysis followed by deposition of pigment in the renal tubules The histological picture of the kidneys also suggested that myanesin may cause cortical ischemia Mallinson (86) reported uremia in two patients who had myanesin during anesthesia He believes that the uremia in these cases and in the case of Hewer and Woolmer was produced by the trauma of the operation and that it belongs to the "crush syndrome without crush injury" picture (85) He described the occurrence of fatal anuria of a similar type in a case anesthetized with thiopental and curare (88)

*Venous thrombosis* Opinion varies as to the cause and frequency of this complication Mallinson (84, 86) and Vartan (125) reported complete freedom from this complication and drew attention to the possibility of pentothal being the cause Stephen and Chandy (117) recorded seven cases of localized thrombophlebitis in a series of 50 administrations All patients recovered within 48 hours Griffith and Cullen (56) had four cases of thrombophlebitis among 120 patients and Musgrove (96) observed this complication in 10 out of his 200 patients Several authors reported single cases of venous thrombosis (126, 55, 134, 39)

*Intra-arterial injection* Ogilvie *et al* (98) described a case of gangrene of the hand and forearm after accidental injection of myanesin Brooks *et al* (26) observed hemolysis in the brachial vein after an injection of the drug into the brachial artery

*Circulatory effects* Hunter (70) saw a simultaneous failure of circulation and respiration of central origin after intravenous injection of 3 grams of myanesin This was at once reversed by 5 cc of nikethamide intravenously Lyall (82)

effect of apo- $\beta$ -erythroidine was similar to that observed after administration of benzimidazole, myanesin or glyketal but differed from these agents in producing paralysis of much longer duration

Apo- $\beta$ -erythroidine did not possess any curare-like action and did not affect two neuron reflexes but depressed selectively multineuron reflexes such as the flexor reflex. It appears that apo- $\beta$ -erythroidine also possesses a selective depressant action on interneurons (107)

#### SPASMOLYTICS

It has been known for over 60 years that certain solanaceous drugs were effective in the treatment of paralysis agitans. The opinion has been expressed that the efficacy of these drugs in certain disorders of the extrapyramidal nervous system was more likely due to their central depressant action than to their inhibiting action on cholinergic nerve endings. With this idea in mind, Domenjoz (39a) examined the pharmacological properties of a number of spasmolytic drugs and recommended parpanit, the diethylaminoethylester of phenylcyclopentanecarboxylic acid for clinical trials in hyperkinetic and dystonic conditions

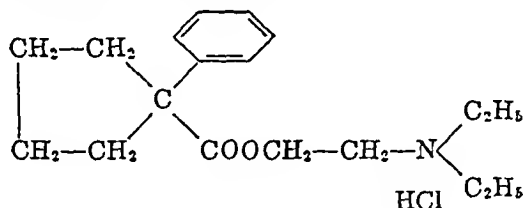


Fig 7 Structural formula of Parpanit

**Pharmacological properties** Parpanit injected in mice or rats in doses of 50 mg per kg caused an increase of spontaneous activity, similar to that observed after comparable doses of atropine. Larger doses of parpanit caused convulsions and death. Paralysis of skeletal muscles did not occur. Parpanit was about 13 times less effective than atropine in antagonizing the action of acetylcholine on the isolated rabbit duodenum but possessed in this respect an activity comparable to other synthetic spasmolytic drugs. Parpanit also had a considerably weaker mydriatic and salivation-inhibiting action than atropine (39a).

In dogs intravenous administration of 3 mg per kg did not markedly affect blood pressure and did not influence the vasodepressor and respiratory reflexes. Larger doses had a depressor effect on the blood pressure and stimulated respiration. Parpanit caused vasodilatation by a direct effect on the vessels and did not act directly on the vasomotor center (66a).

Parpanit did not affect neuromuscular conduction and did not alter excitability of skeletal muscles (66a). Fleisch and Baud (42c), using a quantitative method, found a decrease in the tonus of the adductor muscles of the hind leg of intact and spinal rabbits after parpanit in doses of 4 to 40 mg per kg. They also observed a

knee and ankle jerks. Multineuron reflexes such as the flexor and crossed extensor reflexes were depressed with small doses of the drug. The crossed extensor reflex which is mediated by several interneurons was depressed with smaller doses of glyketal for longer periods of time than the flexor reflex which probably has a shorter intercalated pathway between the afferent and efferent parts of the reflex arc.

Glyketal was very effective in abolishing tremor and repetitive phenomena occurring spontaneously or induced by administration of strychnine. The effects on multineuron reflexes, tremor and repetitive phenomena were apparent after doses of the drug which did not cause paralysis.

The marked effect of the drug on multineuron reflexes and repetitive phenomena, taken in conjunction with its ineffectiveness in suppressing two neuron reflexes and the lack of analgesic action of the drug, suggests that glyketal acts by selectively depressing the interneurons. In this respect, it appeared more potent and selective than benzimidazole and myanesin.

Glyketal did not seem to affect cortical activity and did not influence the electroencephalogram.

*Anticonvulsant action.* Glyketal possessed some antagonistic action to the convulsant and lethal effects of strychnine, metrazol and picrotoxin. This antagonistic action was, however, weak and of a lower order than that of benzimidazole and myanesin.

*Other pharmacological properties.* Glyketal possessed a few other pharmacological actions than those described. It did not cause analgesia in doses not causing paralysis. The sensory impairment during paralysis was probably due to the motor deficit and was not complete. Glyketal had a local anesthetic action comparable to that of procaine.

*Clinical use.* The effect of glyketal in humans has not yet been investigated. It is likely to be of value in conditions in which abnormal impulses reverberate in closed interneuronal circuits such as may be the case in chorea, athetosis, and certain forms of tremor and muscle spasm. It may also be worth a trial in conditions in which the interneurons are in a state of irritation, as may be the case in acute poliomyelitis.

#### APO- $\beta$ -ERYTHROIDINE

It has been suspected for some time that  $\beta$ -erythroidine, apart from its curare-like action, also possesses central depressant properties (30, 61). These two properties have been dissociated in apo- $\beta$ -erythroidine which lacks the peripheral action but still retains the central depressant properties of  $\beta$ -erythroidine (107).

Apo- $\beta$ -erythroidine was prepared by heating  $\beta$ -erythroidine to 120°C with either phosphoric or sulfuric acid. Apo- $\beta$ -erythroidine isolated from the reaction mixture was a solid which, after crystallization from ethanol, melted at 132–132.5°C. It was insoluble in water but could be easily dissolved in dilute acid.

On intraperitoneal administration to mice in doses of 150 mg per kg, apo- $\beta$ -erythroidine caused flaccid paralysis and loss of postural reflexes. The paralyzing

## CHOLINERGIC AGENTS

In view of the potential role played by acetylcholine in the transmission of impulses in the nervous system, its effects on the reflex activity of the spinal cord are of great interest

Schweitzer and Wright (111) found that, in cats under chloralose anesthesia, an intravenous injection of acetylcholine depressed or abolished the knee jerk. This effect was due to a direct inhibitory action on the spinal cord and was only partially annulled by atropine. Neostigmine, carbachol and certain other drugs which prevent the destruction of acetylcholine by cholinesterase behaved similarly. Physostigmine had an opposite effect and caused a marked enhancement of the knee jerk.

Merlis and Lawson (92) investigated the action of physostigmine on spinal reflexes in dogs. They found that it depressed the knee jerk and augmented the flexion and crossed extension reflexes. Bülbbring and Burn (27), using a preparation in which the lower part of the spinal cord and the hind legs were perfused by two different circuits of blood, found that both physostigmine and neostigmine depressed the knee jerk and augmented the flexor reflex. They interpreted their findings by assuming that, in the case of the knee jerk in which only a single synapse is involved, an accumulation of acetylcholine occurs which blocks transmission of the impulse. In the case of the multineuron reflexes, the accumulation of acetylcholine is not great enough to cause blockage at ordinary rates of stimulation, but the muscle fibres contract repeatedly to each single shock, so that a single twitch becomes a short tetanus. It appears that the knee jerk and flexor reflex are modified by eserine and neostigmine as might be expected if transmission at the synapse of the spinal cord would be affected by acetylcholine. This view is further supported by the observation that the effects of both physostigmine on the knee jerk and flexor reflex were prevented or abolished by atropine.

Wikler and Frank (132) studied the effect of neostigmine and physostigmine in chronic spinal dogs. Subcutaneous administration of neostigmine was followed by enhancement of all hindlimb reflexes and the appearance of irregular spontaneous movements of the hindlimbs. Eserine produced spontaneous hindlimb movements which were either irregular or rhythmic. The activity induced by eserine could be abolished by morphine or methadone. The depressant effect of the cholinergic drugs on the knee jerk may have been obscured in these experiments by their direct stimulant action on the muscle (104).

*Clinical use* Neostigmine has had a fairly wide clinical use in neuromuscular dysfunctions in which muscle spasm is an important feature, such as hemiplegia, cerebral palsy, rheumatoid arthritis and subacromial bursitis (74, 33, 45). It has also been used in acute poliomyelitis to reduce pain and relax the muscles (75, 25, 32).

Neostigmine may be of value in these conditions for several reasons. The inhibiting action of the drug on cholinesterase may facilitate the passage of impulses from nerve to muscle by preserving the available acetylcholine at the nerve endings. It may also have a direct action on the synapses in the spinal cord as has

decrease of muscle tonus and an increase in the threshold for the patellar reflex in humans after doses of 100 mg. They believe that parpanit acts on the proprioceptive nerve endings of the muscles and joints. This effect is similar to that of procaine but is stronger and of longer duration.

Parpanit suppressed bradycardia, bronchospasm, hyperactivity of the gastrointestinal tract, convulsions and muscular fasciculations produced by di-isopropyl fluorophosphate (DFP). It also antagonized the convulsive action of strychnine in dogs (66a) but was ineffective in suppressing convulsions caused by nikethamide in guinea pigs (45a).

Berger (7) examined the effect of parpanit on spinal reflexes in cats anesthetized with dial. Small doses of the drug of the order of 2 to 4 mg per kg depressed or abolished the multineuron reflexes, such as the flexor reflex, but did not affect two neuron reflexes such as the knee jerk. This depressant action on interneurons is of interest because parpanit is the only agent of this kind which does not possess paralyzing properties. It may be inferred that the blocking action on interneurons and paralyzing activity are independent properties of drugs, and that paralyzing activity does not constitute a measure of the potential value of a drug as a blocking agent for interneurons.

Gruber *et al* (59) have shown that several autonomic blocking agents inhibiting structures innervated by postganglionic cholinergic nerves also had a depressant action on the central nervous system. They found that parpanit, trasentin and syntropan injected intravenously relieved decerebrate rigidity in cats, and depressed reflexes caused by stimulation of the back and paws in spinal preparations. For these purposes the drugs were found superior to atropine and scopolamine.

*Clinical use* Parpanit has been found of value in the treatment of paralysis agitans and of other dystonic and hyperkinetic conditions (60, 60b, 60c, 21a, 111a). Schwab and Leigh (110a) evaluated quantitatively the effect of parpanit in 50 cases of parkinsonism and found the drug superior to previous medication in 65 per cent of cases. The degree of improvement was usually around 25 per cent. Dunham and Edwards (39b) observed some improvement in 9 out of 19 patients suffering from paralysis agitans. They found the activity of parpanit comparable to that of the solanaceous drugs. Side-actions, in decreasing order of frequency, were giddiness, weakness, drowsiness, paresthesia and rarely hallucinosis. Dryness of the mouth and blurred vision were of lesser severity after parpanit than after atropine.

Parpanit given intravenously in doses of 20 to 30 mg softened the initial contraction and clonic phase of electroshock seizures in humans (60a).

The effect of parpanit on involuntary movements of extrapyramidal origin may be due to the depressant action of the drugs on interneurons. The decrease in rigidity and tremor obtained in certain cases of paralysis agitans is probably not due to the depressant action of the drug on interneurons, because atropine and thephorin (14), which are also of value in parkinsonism, do not affect multineuron reflexes.



it appears likely that anesthetics exert their action specifically on the synaptic region of the motoneurons just as curare does on the endplate region of the muscle (39c)

#### DISCUSSION AND SUMMARY

Most substances possessing a depressant effect on the spinal cord have the important common property of producing paralysis of the ascending type. Tri-*o*-cresyl phosphate, dithiobiuret, benzimidazole, myanesin and glyketal depress the lower segments of the cord in small doses. With larger doses, higher segments of the cord and the midbrain are also affected in an ascending order. The anesthetics differ from the spinal depressants by first depressing higher levels of the central nervous system and by producing paralysis at lower levels only after large doses. The vital functions of the medulla are spared by both the anesthetics and spinal depressants and are affected after toxic doses only.

Whether the spinal depressant action of these agents is direct or is secondary to their effects on certain structures of the midbrain is not known. Clinical observations and the experimental work of Henneman *et al* speak in favor of an indirect action.

The available agents producing depressant effects on the spinal cord may be arbitrarily classified into 4 groups: 1) agents producing irreversible paralysis by damaging the anterior horn cells, such as tri-*o*-cresyl phosphate, 2) agents producing reversible paralysis on chronic administration such as dithiobiuret, 3) agents causing transient paralysis and possessing a selective depressant action on the interneurons, such as the benzimidazole, the glycerol ethers, the 2,2-alkyl-4-hydroxymethyl-1,3-dioxolanes, and apo- $\beta$ -erythroidine, and 4) agents which depress interneurons but do not cause paralysis, such as parpanit.

It is of interest that four chemically different classes of compounds produce similar and highly selective effects on interneurons and postural reflexes. There is no indication that these compounds affect transmission of impulses mediated by acetylcholine. Their mode of action is unknown and when uncovered may bring to light new aspects concerning the transmission of impulses in the central nervous system.

The four families of chemicals blocking interneuronal transmission differ from each other in the intensity of this action and in their antagonism to the effects of strychnine, which to some extent is possessed by all. Glyketal, the most potent interneuronal blocking agent, possesses the weakest anti-strychnine action. Benzimidazole has the strongest antagonistic action to strychnine and the weakest action on interneurons. Myanesin possesses both interneuronal blocking action and anti-strychnine properties to a marked degree. Apo- $\beta$ -erythroidine has a blocking action on interneurons of an order similar to myanesin. Its antagonistic action to strychnine has not yet been investigated.

Spinal depressants and interneuronal blocking agents are of potential therapeutic value in the treatment of muscle spasm, spasticity, tremor and involuntary movement. They may also be of value for the production of muscular relaxation during anesthesia. Up to the present, only myanesin has received adequate clinical

been assumed by Kabat (75), but this effect would be different from that observed by Bülbring and Burn (27) as it was not antagonized by atropine. Neostigmine also possessed a direct stimulant effect on the muscle. This stimulant action was apparent after the cholinesterase was destroyed by di-isopropyl fluorophosphate (104).

The value of neostigmine in spastic conditions is still under discussion and investigation.

#### MORPHINE AND OTHER ANALGESICS

Wikler (131), investigating the action of morphine on the central nervous system of cats, observed in acute and chronic spinal preparations a marked depression of the flexor and crossed extensor reflexes. The knee and ankle jerks were either not affected or were slightly augmented. In long surviving spinal dogs, single doses of morphine or methadone caused similar effects. Luckhardt and Johnson (81), using larger doses of morphine, observed a depressant effect of the drug on the knee jerk of spinal dogs.

It appears that morphine and methadone, in doses comparable to those used in humans, have little effect on two neurons arc reflexes but depress or abolish nociceptive multineuron reflexes. Wikler concluded that these effects may be due to a depressant action of morphine on the interneurons. In view of the strong analgesic action of these drugs, the abolition of responses to nociceptive stimuli may be due to a depressant action of morphine on pain perception. The ineffectiveness of morphine in abolishing reverberating nervous impulses also speaks against a direct action of the drugs on interneurons.

#### BARBITURATES

Beecher *et al* (5) found that the flexor reflex in cats under light barbiturate anesthesia was not followed by an after-discharge. During ether anesthesia, a marked after-discharge was observed. From this they inferred that the long-circuiting of sensory impulses is much more seriously curtailed under barbiturate than under ether anesthesia. Wikler and Frank (132) observed that small doses of pentobarbital sodium of the order of 8 mg per kg abolished the flexor and crossed extensor reflexes in chronic spinal dogs and slightly depressed the knee jerk and ipsilateral extensor thrust. Larger doses of pentobarbital (15 mg per kg) had also a depressant action on the knee jerk and extensor thrust and after still larger doses all reflex activity disappeared.

The barbiturates and other anesthetics cause a progressive depression of all functions of the central nervous system. Eccles (39c) and Brooks and Eccles (25a) have shown that pentobarbital blocks synaptic transmission by so increasing the stability of the surface membrane of the motoneurons that the discharge of impulses is not initiated by normally effective synaptic potentials. Pentobarbital also diminishes the internuncial after-discharge set up by strong stimulation of the dorsal roots, and greatly prolongs the time constant of decay of the dorsal root potential set up by dorsal and ventral root volleys (39d). Although some depression of all components of the reflex pathway has been observed (25a),

21. BLOCH, H Über die Spezifität des Inhibitors bei der Esterase-Hemmung durch Tri-*o*-kresyl phosphat *Helv Chim Acta* 26 733-39, 1943.
24. BRADLEY, W AND BEROER, F M Muscle-relaxing ethers of glycerol. *Nature* 159: 813-14, 1947
25. BRAINERD, H, KATZ, H J, ROWE, JR., A P AND GEIGER, J C Clinical manifestations of poliomyelitis treatment with neostigmine and Kenny method *J Am M A* 123: 718-19, 1945.
- 25a. BROOKS, C McC AND ECCLES, J C A study of the effects of anaesthesia and asphyxia on the mono-synaptic pathway through the spinal cord *J Neurophysiol* 10 349-60 1947
26. BROOKS, D M, HANDFIELD-JONES, E, MACFARLANE R G AND SEDGWY, H J Effects of myanesin on muscles and blood. *Lancet* 1 305, 1948.
27. BÜLERINO E AND BURN, J H Observations bearing on synaptic transmission by acetylcholine in the spinal cord *J Physiol* 100: 337-68, 1941
28. BURKE, J C AND LINEGAR C R. Pharmacological studies of myanesin and curare *Federation Proc.* 7 208, 1948.
29. BURKE, J C, THOMAS, R K, HASSETT JR., G L AND WILKINS, R. J Oral activity of analogues of 3-orthotoxyl 1 2-propanediol *Federation Proc.* 8 277-78, 1949
30. BURMAN M S Clinical experiences with some curare preparations and curare substitutes *J Pharmacol.* 69: 143-48, 1940
31. CARMAN J A Myanesin in anaesthesia *East African M J* 25 184-95, 1948
32. COHEN, A, TROMMER P AND GOLDMAN, J Phenytoin for muscle spasms in rheumatoid arthritis *J Am M A* 130: 265-70, 1946
33. COODLEY, E L. Prostigmine therapy in paralysis *Ann West Med and Surg* 1 425-28, 1947
34. COWEN J Toxic effects of myanesin *Lancet* 1 965, 1948.
35. DALE, L. For and against myanesin *Lancet* 1 651 1948
36. DALE, H W L [Myanesin and haemolysis] *Proc Roy Soc Med* 41 604, 1948.
37. DALLEMAGNE, M J AND PHILIPPOT E Recherches sur la toxicité *l* hexachlorocyclohexane *Arch Int. Pharmacodyn.* 76 274-96 1948
38. DAVIDSON, M H A. Mynoesin as a relaxant in children *Brit M J* 1 544-45 1948
- 38a. DAVIDSON, M H A., WARD A. B AND PASKE E. A Myanesin in the treatment of tetanus *Brit M J* 1 616-18, 1949
39. DINWICK, O P [Myanesin in anaesthetics] *Proc. Roy Soc Med* 41 604 1948
- 39a. DOMENIOS, R. Parpanit, ein neues Therapeutikum beim Störungen der extrapyramidalen Motorik. *Pharmacologie Schweiz. med. Wchnschr* 76 1252-56 1946
- 39b. DUNHAM, W F AND EDWARDS C H Parkinsonism treated with Parpanit *Lancet* 2 724-27, 1945
- 39c. ECCLES J C Synaptic potentials of motoneurons. *J Neurophysiol* 9 87-120 1946
- 39d. ECCLES J C AND MALCOLM J L. Dorsal root potentials of the spinal cord *J Neurophysiol* 9 139-60, 1946
40. EDWARDS, P M Muscle relaxants in clinical practice *Anaesthesia* 3 160-67, 1948.
41. EYDERBY, G E H [Myanesin and haemolysis] *Proc Soc Med* 41 602 1948.
42. FILIPPI E. AND RODOLICO L Proprietà farmacologiche dell' Antodina (feocisopropanediol) *Arch di Farm sper* 11 1-28 1911
- 42a. FINKELMAN I AND ARIEFF A J The action of  $\alpha$   $\beta$ -dihydroxy  $\gamma$  (2 methylphenoxy) propane (Lis-ephen) on peripheral nerves *J Nerv. Ment. Dis* 109 326-29 1949
- 42b. FINKELMAN I AND DUBIN N B Effect of  $\alpha$   $\beta$ -dihydroxy- $\gamma$ -(2 methylphenoxy) propane (Lis-ephen) on excitation of the motor cortex and conduction through the pyramids. *J Nerv. Ment. Dis* 109 323-25 1949
- 42c. FLEISCH, A AND BAUD CH The point of attack of Parpanit *Helv Physiol Acta* 6 331-345, 1948
43. FOSTER, R. H K. Standardization of safety margin *J Pharmacol* 65. 1-17, 1939
44. FOURNEAU E Organic Medicaments and their preparation Translated by W A Silvester London 1925.
45. FOX, M J AND SPANKUS, W H Value of neostigmine in acute anterior poliomyelitis *J Am. M A* 123 720-22, 1945
- 45a. FROMMEL, E., BECK I. T VALLETTE F AND FAVRE, M L'action de l'ester diethyl aminoethylique de l'acide phenylcyclopentane carboxylique Parpanit sur le système nerveux végétatif Son action anesthetique et anticonvulsivante *Arch int. pharmacodyn* 78 379-94 1949
46. GAMMON, G D AND CHURCHILL J A Effects of myanesin upon the central nervous system *Am J M Sc* 217 143-48 1949
47. GILBERT, A. AND DESCOMPS R. Le phénoxypropanediol *Compt Rend Soc. de Biol.* 69 145- 47, 1910
48. GOODKEE, T E W AND GOODHART C E D Fatal snuria after use of myanesin in anaesthesia. *Lancet* 1 183, 1940
49. GOODMAN L. Pharmacodynamic actions of benzimidazole A preliminary report. *Bull. New England M Center* 5: 97-100 1943
50. GOODMAN L. S Personal communications 1948 and 1949
51. GOODMAN L, GILMAN A. AND HART N Preliminary investigations on the pharmacology of benzimidazole *Federation Proc.* 2 80-18 1943
52. GOODMAN L AND HART N Further studies on the central nervous system action of benzimidazole HCl *Federation Proc* 3 73 1944
53. GOODMAN, L S SWINYARD E A AND THOMAS J E P Further studies on the anticonvulsant properties of Tridione (3 5 5-trimethyl-oxazolidine-2 4-dione) *Federation Proc.* 5 179-80 1946.
54. GRAVES E L. ELLIOTT T J AND BRADLEY W Metabolism of  $\alpha$   $\beta$ -dihydroxy  $\gamma$  (2 methyl phenoxy) propane (myanesin) *Nature* 162 257 1948.

cal trials. Although it has produced remarkable effects in numerous cases, the value of the drug is limited. It is unsuitable for routine intravenous use because of the low solubility and the hemolytic action of the drug. The intravenous use of dilute solutions of myanesin during emergencies such as tetanus, strychnine poisoning or status epilepticus may be justified, but intramuscular administration which should be equally effective and less dangerous would be preferable. The oral use of myanesin is impeded by the low potency and rapid inactivation of the drug.

In the past, the activity and potential clinical usefulness of spinal depressants has been evaluated in terms of their paralyzing potency. This criterion of activity is unsuitable because it is not intended to use these agents clinically for the production of paralysis. It appears probable that the interneuronal blocking action or the antagonistic action to strychnine would be a better criterion for assessing the clinical potentialities of these drugs, however, it is not known which of these two unrelated properties is the more important one. This question may be answered by clinical trials of benzimidazole and glyketal, the results of which may indicate new approaches toward the development of more effective agents of this type.

#### REFERENCES

1. ALTSCHUL, S. Effects of dithioburet on the central nervous system. *Proc Soc Exper Med* 66: 448-51, 1947.
2. ASTWOOD, E. B., HUGHES, A. M., LUBIN, M., VANDERLAAN, W. P. AND ADAMS, R. D. Reversible paralysis of motor function in rats from chronic administration of dithioburet. *Science* 102: 196-97, 1945.
- 2a. BAIN, F. L. Impiego della mianesina in anestesia. *Policlinico* 55: ser. prat. 901-905, 1948.
3. BALLANTINE, R. I. W. Myanesin. A report on seventy-six cases. *Anaesthesia* 3: 20-23, 1948.
4. BATE SMITH, E. C. AND BENDALL, J. R. Delayed onset of rigor mortis after administration of myanesin. *J Physiol.* 107: 2P, 1948.
5. BECHER, H. K., McDONOUGH, F. K. AND FORRES, A. Similarity of effects of barbiturate anesthesia and spinal transection. *J Neurophysiol* 2: 81-88, 1939.
6. BELFRAGE, D. H. Tubocurarine in tetanus. *Lancet*, 2: 859-90, 1947.
7. BERGER, F. M. Unpublished observations 1946 to 1949.
8. BERGER, F. M. Effect of anaesthetic and other substances on paralyzing action of myanesin. *Quart J Pharm & Pharmacol* 20: 94-99, 1947.
9. BERGER, F. M. Mode of action of myanesin. *Brit J Pharmacol* 2: 241-50, 1947.
10. BERGER, F. M. For and against myanesin. *Lancet* 1: 651, 1948.
11. BERGER, F. M. Relationship between chemical structure and central depressant action of  $\alpha$ -substituted ethers of glycerol. *J Pharmacol* 93: 470-81, 1948.
12. BERGER, F. M. Paralyzing action of 2-methyl, 2-n-amyl 4-hydroxymethyl 1, 3-dioxolane (glyketal). *Federation Proc* 8: 274, 1949.
13. BERGER, F. M. The pharmacological properties of 2-methyl, 2-n-amyl 4-hydroxymethyl 1, 3-dioxolane (glyketal) a new blocking agent of interneurons. *J Pharmacol* 95: 213-23, 1949.
14. BERGER, F. M. The treatment of paralysis agitans with Thebophorin. *New York State J Med* 49: 1817-20, 1949.
- 14a. BERGER, F. M. The anticonvulsant action of 2-substituted 1, 3-propanediols. *Proc Soc. Exper Med* 71: 270-71, 1949.
15. BERGER, F. M., BOCKELHEIME, V. AND TARBELL, D. S. Pharmacological properties of some 2-substituted-4-hydroxy methyl 1, 3-dioxolanes. *Science* 108: 551-52, 1948.
16. BERGER, F. M., AND BRADLEY, W. Pharmacological properties of  $\alpha$ - $\beta$ -dihydroxy- $\gamma$ -(2-methylphenoxy)-propane (myanesin). *British J Pharmacol* 1: 765-72, 1946.
17. BERGER, F. M., AND BRADLEY, W. Muscle-relaxing action of myanesin. *Lancet* 1: 97, 1947.
18. BERGER, F. M. AND HERZ, E. R. Unpublished observations 1947.
19. BERGER, F. M. AND RILEY, R. F. Preparation and pharmacological properties of the acid succinate of myanesin. *J Pharmacol* 95: 269-75, 1949.
20. BERGER, F. M. AND SCHWARTZ, R. P. Toxicity and muscular effect of d-tubocurarine combined with  $\beta$ -erythroidine, myanesin or eupal J. *Pharmacol* 93: 352-57, 1948.
21. BERGER, F. M. AND SCHWARTZ, R. P. Oral myanesin in treatment of spastic and hyperkinetic disorders. *J A. M. A* 137: 772-74, 1948.
- 21a. BUCKEL, G. AND DUROIS, J. P. Le Parpanit dans le traitement des tremblements de type intentionnel. *Revue Médicale de la Suisse* 67: 708-16, 1947.
22. BISTRÖM, O. AND VIHKARI, S. Glycresin as muscle relaxant in combined anesthesia. *Nord Med* 41: 109-111, 1949.

87. MALLINSON, F B [Myanesin and haemoglobinuria]. *Proc. Roy. Soc. Med.* 41. 635, 1948.
88. MALLINSON, F B Uræmia following anaesthesia. *Lancet* 1 416, 1949.
89. MARSHALL, S V Relaxing agents in anaesthesia: a brief appraisal. *M J Australia* 1 150-53, 1949.
90. MARSTON, A D Current therapeutics II Curare and curare-like drugs (myanesin) *Practitioner* 169 159, 1949.
91. MATHER, L. Myanesin. *Brit. M J* 1 320, 1948.
92. MERLIS, J K and LAWSON, H Effect of curine on spinal reflexes in the dog. *J. Neurophysiol.* 2 575-72, 1939.
93. MORCH, P a Glyceryl-o-crotyl ether Arch. *Pharm. Chem.* 54 327, 1947.
94. MORRISON, J L, RICHARDSON, A P and WALKER, H A Rate of disappearance from the blood and distribution in tissues of 3-(orthotoloxyl)-1,2-propanediol (myanesin) *Federation Proc.* 7 246-47, 1948.
95. MORRISON, J L, SAFERSTEIN, P, WALKER, H A and RICHARDSON, A P Distribution and rate of disposition of myanesin in dogs. *Proc. Soc. Exper. Biol. and Med.* 68 332-43, 1948.
96. MUCONOV, A H Myanesin anaesthesia. *Lancet* 3 163-72, 1949.
97. NOSLE, C B Myanesin anaesthesia. *Lancet* 1 192, 1949.
98. OOLVIE, T A., PENTOLD, J B and CLENDON, D R T Gangrene following intra-arterial injection of myanesin, with a study of blood and myanesin mixtures. *Lancet* 1 947-49, 1949.
99. OOSTENDE, VAN DEN A Sur la pharmacologie de différentes substances curarisantes. *Arch. int. Pharmacodyn.* 75 419-21, 1949.
100. ORLOFF, M J, WILLIAMS, H L. and PFIZFER, C C Timed intravenous infusion of metrazol and strychnine for testing anticonvulsant drugs. *Proc. Soc. Exper. Biol. and Med.* 70 254-57, 1949.
101. PICK, E. P and RICHARDS, G V A biological method for determination of curare and erythrodine-alkaloids. *Proc. Soc. Exper. Biol. and Med.* 67 323-31, 1948.
102. PUOH, J L and ENDERBY, G E H. Haemoglobinuria after intravenous myanesin. *Lancet* 2 387-88, 1947.
103. RICHARDSON, A P, MORRISON, J L. and WALKER, H A. Pharmacologic studies on myanesin. *Am. J. Med.* 4. 455, 1948.
104. RIEKER, JR., W F and WILCOX, W C The Direct Action of Prostigmine on skeletal muscle, its relationship to the choline esters. *J. Pharmacol.* 88 53-66, 1946.
105. RILEY, R. F and BERGER, F M Metabolism of myanesin (3 (o-toloxyl)-1,2-propanediol) *Arch. Biochem.* 20 152-60, 1949.
106. RILEY, R. F and BERGER, F M Preparation and pharmacological properties of the acid succinate of myanesin. *Federation Proc.* 8 323, 1949.
107. SAUVAGE, G L, BERGER, F M and BOECKELHEIDE, V The conversion of  $\beta$ -erythroidine to derivatives of the demethoxy series and some pharmacological properties of apo- $\beta$ -erythroidine. *Science* 109 6-7-20, 1949.
108. SCHLESINGER, E B Pharmacological agents in the treatment of neuromuscular disorders. *Physiotherapy Rev.* 23 100-102, 1948.
109. SCHLESINGER, E B Clinical applications of myanesin. *Trans. New York Acad. Sci.* 11 5-9, 1948.
110. SCHLESINGER, E B, DREW, A L, and WOOD, B Clinical studies in the use of myanesin. *Am. J. Med.* 4. 365-72, 1948.
- 110a. SCHWAB, R. S and LEIGH, D Parapant in the Treatment of Parkinson's disease. *J. Am. M. A.* 149 629-34, 1949.
- 110b. SCHWAB, R. S and UNVA, H. R. Some effects of myanesin in psychiatric patients. *J. Am. M. A.* 140 672-73, 1949.
111. SCHWITZER, A. and WRIGHT, S Further observations on the action of acetylcholine, prostigmine and related substances on the knee jerk. *J. Physiol.* 89 384-402, 1937.
- 111a. SIMMA, K. Über die Wirkungsweise des Parapants bei extrapyramidal motorischen Störungen. *Monatsschr. f. Psychiat. u. Neurol.* 114. 119-125, 1947.
112. SMITH, H W Comparison of racemic and levo glyceryl-guaciscol ethers for anesthetic and other actions. *J. Pharmacol.* 91. 93-97, 1947.
113. SMITH, M I, ELVOE, E and FRAZIER, W H Pharmacological action of certain phenol esters, with special reference to the etiology of so-called ginger paralysis. *Pub. Health Rep.* 45 2509-24, 1920.
114. SMITH, M I, ELVOE, E, VALAER, P J, FRAZIER, W H and MALLORY, G E. Pharmacological and chemical studies of the cause of so-called ginger paralysis. *Pub. Health Rep.* 45. 1703-16, 1920.
115. SMITH, M I, ENGEL, E W and STOHLMAN, E F Further studies on the pharmacology of certain phenol esters with special reference to the relation of chemical constitution and physiologic action. *Nat. Inst. Health Bull.* No. 160 p 1-69, 1932.
116. SMITH, M I and LILLIE, R. D. Histopathology of triorthocresyl phosphate poisoning. Etiology of so-called ginger paralysis (third report). *Arch. Neurol. Psychiat.* 26 976-92, 1931.
117. STEPHEN, C R and CHANDY, J Clinical and experimental studies with myanesin (preliminary report). *Canad. Med. Ass. J.* 57 463-63, 1947.
118. TITUS, E., ULICK, S and RICHARDSON, A. P The determination of 3-(orthotoloxyl)-1,2-propanediol (myanesin) in body fluids and tissues, and its disappearance from the blood following intravenous injections in the dog. *J. Pharmacol.* 93 129-34, 1949.
- 118a. TOMAN, J E P and EVERETT, G Personal communication 1949.
119. TOMAN, J E P, GOODMAN, L. S and SWINYARD, E A Observations on the central excitatory effects of metrazol. *Federation Proc.* 5 203, 1946.
120. TOMAN, J E P, SWINYARD, E A and GOODMAN, L. S A comparison of time relations in convulsive and non-convulsive responses to cortical stimulation. *Federation Proc.* 5: 105-108, 1946.
121. TOMAN, J E P, SWINYARD, E A and GOODMAN, L. S Properties of maximal seizures, and their alteration by anticonvulsant drugs and other agents. *J. Neurophysiol.* 9 231-39, 1946.
122. TORRENS, J A, EDWARDS, P M and WOOD, M W W Myanesin in tetanus. *Lancet* 2. 807-809, 1948.
123. TURNBULL, T A. Myanesin, *J. Roy. Nav. M. Serv.* 33. 41-42, 1947.

- 65 GRAY, T C [Myanescin and haemolysis] *Proc Roy Soc Med* 41: 602-604, 1948.
- 66 GRIFFITH, H R AND CULLEN, W G Myanescin as a muscle relaxant *Anesth & Analg* 27: 232-35, 1948.
- 67 GRIFFITH, H R AND JOHNSON, G E Use of curare in general anesthesia *Anesthesiology* 3: 418-20, 1942
- 68 GRIFFITH, H R, STEPHEN, C R, CULLEN, W G AND BOURNE, W Myanescin as a muscle relaxant. *Anesthesiology* 10: 81-85, 1949
- 68a. GROSS, E AND GROSSE, A Ein Beitrag zur Toxikologie des Orthotriäcetylphosphates *Arch exper Path. & Pharmacol* 168: 473-514, 1932
- 69 GRUBER, C M, KRAATZ, C P, GRUBER JR., C M AND CHPELAND, J E Effect of diethylaminocetyl ester of 1 phenyl cyclo-pentano-1-carboxylic acid by drochloride, parpanit, on decerebrate rigidity, spinal reflexes and skeletal muscle *Federation Proc* 8: 297, 1949
- 69a GRUNTHAL, E Ueber Parpanit, einen neuen, extrapyramidal motorische Störungen beeinflussenden Stoff *Schweiz Med Wchnschr* 76: 1288-89, 1948
- 69b GRUNTHAL, E AND UNVARNELLY, G Über die Einwirkung intravenöser Parpanitgaben auf den Krampfanfall beim Elektroschock *Monatsschr f Psychiat u Neurol* 115: 223-232, 1948
- 69c HARTMANN, K Erfahrungen mit dem neuen Präparat Parpanit bei der Behandlung von Erkrankungen des extrapyramidalen motorischen Systems *Schweiz Med Wchnschr* 76: 1289-91, 1948
- 69d HARTMANN, K The use of Parpanit in neurological cases *Therap Rev* 3 (pt 2) 163-65, 1947
- 70 HARVEY, A M AND MARLAND R S Actions of curarizing preparations in the human *J Pharmacol* 73: 304-11, 1941
- 71 HAY, J Observations on the use of myanescin in psychiatric cases *Canad Med Ass J* 60: 224-27, 1949
- 72 HENNINGMAN, E KAPLAN, A AND UYMA, K Effect of myanescin on extrapyramidal facilitatory and inhibitory systems *Federation Proc* 8: 301-2, 1949
- 73 HEWER, A J H Anesthetics [Use of myanescin in cats] *Proc Roy Soc Med* 41: 603, 1948.
- 74 HEWER, T F AND WOLLMER, R F Death from renal anoxia after myanescin anaesthesia. *Lancet* 2: 604-10, 1947
- 75 HEWER, T F AND WOLLMER, R F Death after myanescin anaesthesia *Lancet* 1: 117, 1948
- 76a HEYMANS, C AND VLEESCHOUWER, G R. De Actions pharmacologiques de l'ester diethylaminocetyllique de l'acide phenyl-cyclopentano-carboxylique (Parpanit) *Arch Internat. de pharmacodyn et de therap.* 75: 307-324, 1948.
- 77 HINE, C H AND CHRISTENSEN, H E Comparative activity of some  $\alpha$  substituted glycerol and glycidyl ethers. *Federation Proc* 7: 227, 1948
- 78 HODGE, H C AND STERNER, J H Skin absorption of triorthocresyl phosphate as shown by radioactive phosphorus *J Pharmacol* 79: 225-34, 1943
- 78a HOLT, W L JR., GREENBLATT, M AND ANDERSON, R Myanescin in electric shock, studies in rabbits and man *Am J Psychiat* 105: 834-39, 1948-49
- 78b HOLT, W L JR., RINKEL, M., GREENBLATT, M AND ANDERSON, R Myanescin effects in rabbits undergoing electric shock *J Nerv & Ment Diseases* 109: 271, 1949
- 79 HUNTER, G Diazo reaction in urine *Biochem J* 19: 25-33, 1925
- 80 HUNTER, A R [Myanescin in hyperkinetic states] *Proc Roy Soc Med* 41: 603, 1948
- 81 HUNTER, A R AND WATERFALL J M Myanescin in hyperkinetic states *Lancet* 1: 366, 1948
- 82 HUTTON J N T "Myanescin" as a relaxant in children *Brit M J* 1: 757 1948
- 82a ITALIE, VAN L., HARMATA, A AND EVELD, VAN L W Die Untersuchung von Abortiva, besonders "Apio!" *Arch exper Path & Pharmacol* 165: 84-100 1932
- 83 KAADA, B R Site of action of myanescin (tolserol) in the central nervous system *Federation Proc* 8: 53, 1949
- 84 KABAT, H Studies on neuromuscular dysfunction I Neostigmine therapy of neuromuscular dysfunction resulting from trauma II Neostigmine therapy of hemiplegia facial paralysis and cerebral palsy III Neostigmine therapy of chronic rheumatoid arthritis and subacromial bursitis *Pub Health Rep* 59: 1635-51, 1949
- 85 KABAT, H AND KNAF, M E Use of prostigmine in the treatment of poliomyelitis *J Am M A* 122: 989-95, 1943
- 86 KINN, J G AND LANGWORTHY, O R Jake paralysis Paralysis following the ingestion of Jamaica ginger extract adulterated with tri-ortho-cresyl phosphate *Bull Johns Hopkins Hosp* 52: 39-60 1933
- 87 L L Utilization pour l'anesthésie d'un nouvel agent curariant de synthèse *Presse Méd* 1: 348, 1947
- 88 LAUNOY, L Sur le phénoxypropanediol *Compt Rend Soc Biol Paris* 69: 191-92, 1910
- 89 LILLIE, R D AND SMITH, M I The Histo-pathology of some neurotoxic phenol esters *Nat Inst Health Bull.* No 160 54-62, 1932
- 89a LOEB, P C Comparative toxicities of five substituted glycerol ethers *Federation Proc* 8: 316, 1949
- 90 LOTT, W A Historical introduction, with some general comments concerning the relation between chemical structure and relaxant activity *Trans New York Acad Sci* 11: 2-5 1948
- 91 LUCKHART A B AND JOHNSON, C A Studies on knee jerk effect of moderate doses of morphine sulphate on reflex excitability of spinal cord *Am J Physiol* 83: 634-41 1928
- 92 LYALL, A Myanescin in anaesthesia, observations of 250 cases *Glasgow med J* 28: 313-19, 1947
- 92a. MACAR, J Essai sur la myanescine *Acta Chir Belg* 48: 29-49, 1949
- 93 MALLINSON, F B A new synthetic curarizing agent in anaesthesia *Lancet* 1: 98-100, 1947
- 94 MALLINSON, F B Some general aspects of the technique of curarization *Proc Roy Soc Med* 40: 588-92, 1947
- 95 MALLINSON, F B Death after myanescin anaesthesia *Lancet* 1: 41, 1948.
- 96 MALLINSON, F B The present position of myanescin in anaesthesia, clinical aspects *Proc Roy Soc. Med* 41: 603-605, 1948.

# THE PHARMACOLOGICAL ACTIVITY OF EPINEPHRINE AND RELATED DIHYDROXYPHENYLALKYLAMINES

A M LANDS

*Sterling-Winthrop Research Institute, Rensselaer, New York*

The pharmacology of sympathomimetic amines has been the subject of several previous reviews (33, 116, 117, 247) The large volume of published research now available makes it advisable to limit this review to a part of the entire subject

An attempt has been made to present here the pharmacological actions of epinephrine and the structurally related dihydroxyphenylalkylamine derivatives on smooth and cardiac muscle The publications cited must be considered merely as representative of this field of investigation The principal compounds described are identified by the commonly used names

EPINEPHRINE, 'ADRENALIN', 'SUPRARENIN', LEVO 1-(3',4'-DIHYDROXY-PHENYL)-2-METHYLAMINOETHANOL

Earlier publications have reviewed the isolation, structural determination and synthesis of epinephrine (116, 247) A marked rise in blood pressure following the intravenous injection of extracts of the adrenal glands was demonstrated in 1895 (193), and shortly thereafter the similarity of this response to the effects produced by stimulation of sympathetic nerves was described (36, 77, 154, 159) More recent investigations have indicated that epinephrine-like substances may be produced in many organs

The intravenous injection of small amounts (1-5 mcg /kgm ) of epinephrine (63, 77) causes a prompt rise in blood pressure The resulting rise has been divided into 4 phases as follows, (a) vasoconstriction, (b) decremental phase of (a), (c) a secondary rise associated with stimulation of the vasomotor center, (d) a fall below the preinjection level A similar analysis by the 3 manometer method of Nolf has shown (a) a marked increase in stroke volume associated with a distinct increase in heart action, (b) a fall in blood pressure induced by the sinus reflex mechanism, (c) a diminution in the output of the heart, partly reflex in origin and partly the result of diminished venous return, (d) a period in which peripheral actions are dominant and wherein there is an increased stroke volume due to an increased venous return associated with constriction of capillaries and veins and (e) a fall in pressure as the peripheral action diminishes (215) The fall in pressure below the preinjection level which follows the pressor response (182) probably results from the vasodepressor action of epinephrine (51) or from reflex vasodilatation (119) There is a marked, but transient, increase in the output of the heart coincident with an increase in heart rate (22, 99), which takes place within a few seconds after intravenous injection This rise precedes the peripheral vasoconstriction which in turn tends to diminish cardiac output (22) The sudden increase in arterial pressure associated with the increase in cardiac output stimu-

- 124 UNNA, K AND KAPLAN, A Anticonvulsive properties of myanesin Federation Proc 8 341, 1949
- 125 VARTAN, K For and against myanesin Lancet 1 613, 1948.
- 126 VARTAN, K For and against myanesin Lancet 2: 73, 1948
- 127 VOLTERRA, M Urinary phenols method of determination Am J Clin Path 12 625-33 1942
- 128 WALKER, H A , RICHARDSON, A P , LOZN, P AND PERON J The paralytic and lethal action of myanesin, pentobarbital and combinations of these agents in mice Federation Proc 7: 262, 1948
- 129 WELCH, H AND HUNTER, A C Method for determining the effect of chemical antiseptics on phagocytosis Am J Pub Health 30 129-37, 1940
- 130 WHITEHEAD, R. G Myanesin A review of the literature Manitoba Med Rev 28 644-45, 1948.
- 131 WIKLER, A Studies on action of morphine on central nervous system of cat J Pharmacol 80 176-87, 1944.
- 132 WIKLER, A AND FRANK, J Hindlimb reflexes of chronic spinal dogs during cycles of addiction to morphine and methadon J Pharmacol 94 382-400 1948
- 133 WILSON, H B [Use of myanesin in anesthesia] Proc Roy Soc Med 41 605, 1948.
- 134 WILSON, H B AND GORDON, H E Myanesin as an aid to anaesthesia in children Lancet 1 367, 1948.
- 134a WOOLEY, D W Some biological effects produced by benzimidazole and their reversal by purines J Biol Chem 152: 225-32, 1944
- 135 WOOLMER, R [Muscle-relaxing effect of myanesin] Proc Roy Soc Med 41 604, 1948.
- 136 WYNGAARDEN, J B , WOODS, L A AND SEEVERS, M H Plasma levels and urinary excretion of injected myanesin in dogs Proc Soc Exper Biol and Med 66: 258-60, 1947



the vagi above the heart (37, 128, 184) A similar reflex slowing of the heart, associated with an increase in pressure, which may be prevented by atropine has been reported in man (88, 90) Slow intravenous infusion (10 mcg /kg /min ) in man has been observed to increase cardiac output without an increase in venous pressure or heart rate (183)

The determination of the effect of epinephrine on coronary flow is made difficult by the mechanical factors involved An increase has been reported for saline perfused beating hearts (21, 35, 77, 105) Determinations in the heart-lung preparation of dogs indicate that epinephrine causes an increase in coronary flow (174), this is also observed when heart rate and total output are maintained constant (10) Very small amounts (0.1 mcg) have been reported to diminish coronary flow without change in cardiac action, whereas larger doses (10 mcg or more) caused transient constriction followed by dilatation (174) Coronary flow was decreased by epinephrine in the human heart-lung preparation (145), this reduction was associated with an increased vigor and rate of contraction In the non-beating human heart (145) and in cat, rabbit and dog hearts (137), an increased flow was observed Isolated rings of coronary arteries of the ox (153) and sheep (60) are relaxed by epinephrine whereas those from man are reported to contract with dilutions greater than 1:50,000 and to relax at higher concentrations (145) Stromuhr determination of coronary flow has revealed a decrease during systole but an increase during diastolic inflow (100, 207) Flow determinations in unanesthetized dogs have indicated that epinephrine causes a distinct increase, the increase being relatively greater than would be expected from the increase in mean blood pressure (253)

The determination of the effect of epinephrine on pulmonary blood flow is also made difficult by the simultaneous stimulating action on the heart Experiments with isolated strips of pulmonary arteries obtained from various species of animals and from man have indicated that epinephrine causes constriction (20, 44, 60, 170, 186) Rings taken from the extrapulmonic arteries respond to dilutions of 1:1 million to 1:10 million by constriction Intrapulmonic arteries gave small and variable responses but with constriction predominating (87, 109, 208) Constriction has also been reported for the perfused pulmonary vascular bed (44, 91, 198, 249, 256) The various results obtained have been described in a recent summary (68)

An increase in right along with a decrease in left auricular pressure has been observed following intravenous epinephrine injection and has been interpreted as an indication of pulmonary vasoconstriction (198, 257) However, such results could be expected to follow an increased output from the heart, as described earlier in this article Numerous experiments indicate that epinephrine can cause pulmonary vasoconstriction (68, 212) but it seems doubtful if this plays an important role in the regulation of pulmonary blood flow (135)

Epinephrine is an effective bronchodilator drug This action has been demonstrated on excised tracheal tissue (55, 172, 245, 246) and on the isolated perfused lung (233, 240, 252) when contraction had been induced by histamine, barium chloride and other bronchoconstrictor agents Experimentally induced bronchospasm in cats and dogs is readily relaxed by small intravenous doses (0.01-

lates pressoreceptors in the aorta and carotid sinus and thereby reflexly induces some vasodilation (119)

The peripheral effects of epinephrine are quite variable in different organs and in the same organ under varying physiological conditions. Epinephrine usually causes constriction in the cutaneous vascular bed (58, 98, 127). Small amounts of epinephrine may cause an increase (66, 98, 114, 129) followed by a decrease (199) or a primary decrease (67, 199) in blood flow through muscles. Slow intravenous infusion causes an increase in blood pressure along with a marked increase in the volume flowing through the limbs of experimental animals and man (5, 130). Limb volume and venous outflow studies in the hind limbs of dogs after the removal of the skin reveal only vasodilation after epinephrine whereas in the intact leg the net effect is usually a reduction in volume due to the marked constriction of the skin vessels (127). In anesthetized cats, intravenous injections of 0.5 mcg\*/kg/min caused a marked increase in the blood flow through the hind limb but this increase diminished as the rate of injection was increased (102). A decrease in the rate of flow through bones has been observed with epinephrine in perfusion experiments (74).

Epinephrine frequently increases the volume of abdominal organs (75, 126) and increases the volume flow (5, 19, 75, 126, 192). However, constriction of mesenteric vessels has been reported (44, 58, 83, 113, 129, 173, 192). Small doses of epinephrine may cause an increase in portal flow and liver volume with a reduction in venous pressure (19, 27, 40, 75, 97, 138, 217), whereas large doses cause a reduction in both hepatic flow and liver volume (19, 27, 40, 75, 97, 138, 173, 180, 217). Epinephrine causes contraction of the splenic capsule (129, 211). An initial contraction followed by marked relaxation may occur (129).

Subcutaneous or intravenous injection of epinephrine in man causes tachycardia, an increase in systolic and usually a decrease in diastolic pressure associated with a reduction in peripheral resistance (5, 88, 90, 94, 201). The determination of cardiac output, pulse pressure and total peripheral resistance during slow intravenous infusion has led to the conclusion that, in man, epinephrine is predominantly a vasodilator agent (5, 94).

Epinephrine is a potent cardiac stimulant. Isolated frog heart preparations respond to dilutions of 1:10 million to 1:1 million by an increase in both rate and amplitude of contraction. Greater concentrations cause arrhythmia and cardiac arrest (1, 9, 112, 162, 169). Similarly, mammalian heart strips, excised mammalian hearts and heart-lung preparations respond to epinephrine by an increase in rate and force of contraction associated with an increased oxygen consumption (59, 96, 118). Epinephrine causes a reduction in the length of systole and shifts the time of maximum ejection into the early part of systole. There is an increase in contractile activity and this may result in increases in stroke volume and cardiac output if peripheral resistance is not too greatly increased. Doses which markedly increase peripheral resistance also increase diastolic volume and reduce cardiac output and work (110, 204). In the intact animal, there may be a reduction in the rate of contraction which is prevented by atropine or by cutting

articles cited (33, 104, 247) Epinephrine has been reported to increase the force of uterine contractions during delivery, this effect being followed by a short period of diminished activity (258)

The isolated urinary bladder of the cat is contracted by small and relaxed by large concentrations of epinephrine (76) Intravenous injection causes a transient contraction which may be followed by a reduction in tonus (159) Activity of the ureter of the cat is increased and the urethra is strongly contracted (78, 166) The dog bladder usually responds by a small increase in tonus (78) The rabbit bladder may be weakly contracted or may relax (2), whereas the human bladder is contracted and rhythmic activity of the ureters is increased by epinephrine (171, 222)

TABLE 2  
*The acute toxicity of epinephrine*

ANIMAL	ADMINISTERED	TOXIC DOSE mg/kg	REFERENCE
Mouse	i v	2.7 ± 0.2 (LD <sub>50</sub> )	123
	i p	4.6 ± 0.55 (LD <sub>50</sub> )	149
	s c	1.0-1.5	33, 80
		1.98-2.17	157
		4.0-8.0	221, 247
	oral	50.0	33
Rat	i v	0.04 ± 0.004 (LD <sub>50</sub> )	123
		0.005-0.05	33
	s c	5.0-10.0	33
		10.0-20.0	247
	oral	30.0	33
Rabbit	i v	0.2-0.3	33
		0.05-0.4	32, 82, 156
	s c	10.0-20.0	33
		4.0-10.0	26, 247
	oral	30.0	33
Guinea pig	i v	0.15-0.2	157, 216
Cat	i v	0.5-8.0	33, 247
	s c	20.0	33, 247
Dog	i v	0.2-2.0	33, 247
	s c	5.0-6.0	8, 33, 247
Human	i.m.	>7	124, 181

Epinephrine, perfused through the superior cervical sympathetic ganglion of the cat, causes depression of the response to repetitive stimulation of the pre-ganglionic trunk (175) This inhibitory action appears to be similar in nature to the inhibition obtained on the intestine and bronchi (176) Other investigators have reported increased transmission with large doses (38)

Epinephrine is a very toxic drug Data on acute toxicity are summarized in table 2 The symptoms of intoxication in rats following intravenous administration are depression, blanching of the extremities, dyspnea, loss of muscular

0.02 mg/kg) of epinephrine (42, 95, 131, 134, 195, 231) The inhalation of a histamine mist induces marked bronchospasm in guinea pigs This can be prevented by the previous intraperitoneal injection of 0.1 mg/kg of epinephrine (225) Histamine and acetylcholine mists induce bronchoconstriction in guinea pigs which is relaxed by epinephrine (214, 244) The inhalation of an epinephrine mist has also been found effective in inducing bronchodilatation in bronchial asthma (69, 223)

The action of epinephrine on the gastro-intestinal tract varies with the segment studied, with the degree of initial tonus and with the species of animal With cat and human stomachs, the pyloric sphincter is contracted while all other parts are relaxed The preantrum of the dog's stomach is relaxed while the body, fundus and cardiac sphincter are contracted (54, 226) The frog stomach may respond by either contraction or relaxation whereas the turtle stomach is contracted

TABLE 1  
*Effect of epinephrine on the uterus\**

ANIMAL	NON-GRAVID	GRAVID	REFERENCE
Mouse	—	—	104
Rat	—	—	104, 107, 141, 168
Guinea pig	—	—	104, 107, 168, 179
	+	+	60, 104, 139, 149
Rabbit	+	+	33, 104, 149, 168, 247
Cat	—	+	33, 104, 247, 254
Dog	+	+	104, 146
	—	—	104, 139
Monkey	+	+, —	66, 104
Human	+	+	103, 104, 139, 146, 191, 209

\* — inhibitory, + constriction

by epinephrine (29) In anesthetized dogs, small intravenous doses (2.5–20 mcg) of epinephrine diminish gastric tonus (226) Epinephrine stimulates the lower oesophagus and cardia and inhibits the stomach in cats In rabbits, epinephrine inhibits the cardia and contracts the stomach (54)

Tonus and peristalsis in the small intestine are diminished by epinephrine Isolated segments of rabbit intestine are relaxed by dilutions as great as 1:500 million (125) Segments of guinea pig ileum are relaxed by dilutions of 1:20 million but may be contracted by dilutions of 1:1 million to 1:4 million (149) The pyloric (50, 140, 229) and ileocecal (50, 66) sphincters are contracted Marked inhibition of the intestine follows intravenous injection of epinephrine and the duration of this action parallels that of the pressor action (248) Denervation of the small intestine increases its sensitivity to this inhibitory action (260) Epinephrine also causes relaxation of the gall bladder (18, 160)

The action of epinephrine on the uterus is quite variable, being influenced by the condition of the organ (gravid, non-gravid) and by the species of animal used These various actions have been summarized in table 1 and in the review

articles cited (33, 104, 247) Epinephrine has been reported to increase the force of uterine contractions during delivery, this effect being followed by a short period of diminished activity (258)

The isolated urinary bladder of the cat is contracted by small and relaxed by large concentrations of epinephrine (76) Intravenous injection causes a transient contraction which may be followed by a reduction in tonus (159) Activity of the ureter of the cat is increased and the urethra is strongly contracted (78, 166) The dog bladder usually responds by a small increase in tonus (78) The rabbit bladder may be weakly contracted or may relax (2), whereas the human bladder is contracted and rhythmic activity of the ureters is increased by epinephrine (171, 222)

TABLE 2  
*The acute toxicity of epinephrine*

ANIMAL	ADMINISTERED	TOXIC DOSE mg/kg	REFERENCE
Mouse	i v	$2.7 \pm 0.2$ (LD <sub>50</sub> )	123
	i p	$4.6 \pm 0.55$ (LD <sub>50</sub> )	149
	s c	1.0-1.5	33, 80
		1.98-2.17	157
		4.0-8.0	221, 247
	oral	50.0	33
Rat	i v	$0.04 \pm 0.004$ (LD <sub>50</sub> )	123
		0.005-0.05	33
	s c	5.0-10.0	33
		10.0-20.0	247
Rabbit	oral	30.0	33
	i v	0.2-0.3	33
		0.05-0.4	32, 82, 156
	s c	10.0-20.0	33
		4.0-10.0	26, 247
Guinea pig	oral	30.0	33
	i v	0.15-0.2	157, 216
Cat	i v	0.5-8.0	33, 247
	s c	20.0	33, 247
Dog	i v	0.2-2.0	33, 247
	s c	5.0-6.0	8, 33, 247
Human	i.m.	>7	124, 181

Epinephrine, perfused through the superior cervical sympathetic ganglion of the cat, causes depression of the response to repetitive stimulation of the pre-ganglionic trunk (175) This inhibitory action appears to be similar in nature to the inhibition obtained on the intestine and bronchi (176) Other investigators have reported increased transmission with large doses (38)

Epinephrine is a very toxic drug Data on acute toxicity are summarized in table 2 The symptoms of intoxication in rats following intravenous administration are depression, blanching of the extremities, dyspnea, loss of muscular

coordination which may be followed by clonic convulsions and death from respiratory arrest (123) Chloroform (158) and cyclopropane (194) increase the sensitivity of the heart to the toxic actions of epinephrine Accidental epinephrine intoxication in man has been reported (124, 181), with intramuscular doses of 5-7 mg not causing death Symptoms observed were headache, excessive perspiration, a feeling of constriction in the neck with fullness in the chest, precordial distress, mild tremor and vomiting

#### DEXTRO-EPINEPHRINE

The pharmacological responses to the dextro-isomer of epinephrine appear to be qualitatively the same as those obtained with epinephrine (89) The pressor potency of epinephrine has been reported to be 12-51 (64), 20 (234),  $18.5 \pm 0.7$  (232) times greater than that of the *d*-isomer The toxicity of the *d*-isomer is distinctly lower than that of epinephrine It is  $\frac{1}{2}$  -  $\frac{1}{3}$  as toxic as this latter drug when injected subcutaneously in rats (64),  $\frac{1}{15}$  -  $\frac{1}{10}$  as toxic when injected intravenously in mice (123) and  $\frac{1}{2}$  -  $\frac{1}{3}$  as toxic when injected intravenously in rabbits (247)

#### ARTERENOL,\* NOR-EPINEPHRINE, 1-(3',4'-DIHYDROXYPHENYL)-2-AMINOETHANOL

Arterenol was synthesized in 1904 by Stolz and Flächer (230) and by Dakin (65) The racemic compound has recently been resolved by Tullar (250)

The pressor potency of arterenol has been reported to exceed that of epinephrine, arterenol is 1.25-1.5 times more pressor in dogs when the racemic compounds are compared (25, 220) The size of the dose injected may influence the ratio obtained Arterenol and epinephrine are reported to be equipressor at small doses but an increase in the relative potency of arterenol was observed as the dose was increased (179) Arterenol has been reported to be 1.3 times more pressor than epinephrine in cats (179, 237, 254) and the *l*-isomer 1.7 times more pressor than epinephrine in dogs (168) Epinephrine is 1.5-2.5 times more active than arterenol in causing vasoconstriction in the perfused rabbit ear (179) and has also been reported more effective in causing vasoconstriction of the renal, mesenteric and femoral vascular beds after intra-arterial injection (4) An equal increase in coronary flow of the perfused rabbit heart was observed with both arterenol and racemic epinephrine (179) A decrease in coronary flow has also been reported (4)

Arterenol has been reported to be less stimulating than epinephrine on the perfused frog heart (254) Equal effects on rate and amplitude of the perfused rabbit heart (179) have been reported when the racemic salts were compared Equipressor doses of the *l*-isomers were reported to be equally stimulating on the dog heart *in situ* (168) or on the denervated heart (259) In other experiments, arterenol has been reported to be 1.7 times more stimulating than epinephrine (62)

The effects of intravenous infusion of 10-20 mcg/kg/min *l*-arterenol for 3

\* Arterenol and other optically active sympathomimetic amines are referred to as the racemic compound unless otherwise designated Epinephrine is the *l* base

minutes in 7 normal adults were compared with those obtained with this dose of epinephrine. Arterenol caused bradycardia, whereas epinephrine caused tachycardia. The subjective effects of arterenol were insignificant whereas with epinephrine there was mild palpitation, hyperventilation, tightness in the chest and muscular fatigue (23). Intravenous infusion of lesser amounts (0.11–0.4 mcg/kg/min) of *l*-arterenol for 14 to 22 minutes caused either no change or a decrease in cardiac output, a significant rise of systolic and diastolic arterial pressures with a rise of mean pressure, an increase in total peripheral resistance, a decrease in pulse rate and a significant rise in mean pulmonary arterial pressure (94).

Isolated segments of small intestine are relaxed by arterenol. The relative activity varies with the species of animal used, the inhibitory potency of epinephrine is reported to be equal to that of arterenol in the cat, but greater in the rabbit and rat (4, 79, 168, 179). Racemic epinephrine and arterenol have been reported to be equally active on the isolated guinea pig ileum (179). Epinephrine was found to be 1.4 times more active than arterenol when the *l*-isomers of both compounds were used (168).

The isolated cat uterus is inhibited by arterenol, but less readily than by epinephrine (25). Inhibition of the isolated guinea pig uterus requires 2.5–10 times more arterenol than epinephrine (25, 149, 169). The isolated rat uterus, stimulated with acetylcholine, is relaxed by *l*-arterenol in a dilution of 1:10 million–1:3 million or at concentrations 30 times greater than the effective concentration of epinephrine (168).

The bronchioles of the isolated perfused guinea pig lung are dilated by racemic arterenol in doses 7–17 times greater than the effective dose of epinephrine (168, 179, 240). Bronchoconstriction, induced in unanesthetized guinea pigs by inhalation of histamine mists, may be prevented or diminished by the parenteral administration of arterenol. However, the dose required is about 3 times greater than that required for epinephrine (168). Studies in pithed dogs have shown epinephrine to be 7.4 times more active than arterenol in relaxing bronchoconstriction induced by arecoline (195). Histamine-induced bronchospasm, in this preparation, was found to be more readily relaxed by epinephrine, arterenol being only moderately effective in producing bronchodilatation (42).

The central nervous system stimulation of arterenol and epinephrine was compared in rats. Epinephrine was found to be about twice as stimulating as *l*-arterenol (168). This is somewhat at variance with an earlier report which indicated no central nervous system stimulation from *dl*-arterenol (219).

The acute toxicity of arterenol is somewhat less than that of epinephrine. The various results obtained are shown in table 3 (p. 286).

The pharmacologic activity of the *d*-isomer of arterenol is qualitatively the same as that of the *l*-isomer but the potency is much lower. The relative potencies of the *d*-isomer, expressed as multiples of the effective dose of the *l*-isomer, have been reported as follows: vasopressor and cardiac action—27, inhibition of the guinea pig ileum—62, inhibition of the isolated rat uterus—4, dilatation of the bronchioles of the isolated perfused guinea pig lung—50, 60, bronchodilatation by the histamine mist method—20 (168).

The acute intravenous toxicity of *d*-arterenol in mice and rats is shown in table 3 The *d*-isomer is distinctly less toxic than the *l*-isomer

### HYDROXYTYRAMINE, 1-(3',4'-DIHYDROXYPHENYL)-2-AMINOETHANE

The pressor action of hydroxytyramine is much less than that of arterenol or epinephrine Results obtained on cats and dogs suggest that it is  $\frac{1}{8}$ – $\frac{1}{4}$  as potent as epinephrine (6, 25, 108, 235) Action on the dog heart-lung preparation and on the isolated perfused cat heart indicates a potency of about  $\frac{1}{8}$  that of epinephrine

TABLE 3  
*The acute toxicity of arterenol*

ANIMAL	DRUG	ADMINISTERED	TOXIC DOSE	REFERENCE
			mg/kg	
Mouse	levo	i v	5.0 $\pm$ 1.0 (LD <sub>50</sub> )	123
	racemic	i v	7.5 $\pm$ 2.0 (LD <sub>50</sub> )	123
	racemic	i v	9.8 $\pm$ 1.9 (LD <sub>50</sub> )	71
	racemic	i v	5.0 (LD <sub>50-90</sub> )	179
	dextro	i v	60.0 $\pm$ 20.0 (LD <sub>50</sub> )	123
	racemic	i p	12.0 – 30.0 (LD <sub>50-90</sub> )	179
	racemic	i p	15.6 $\pm$ 3.8 (LD <sub>50</sub> )	71, 149
	racemic	s c	40.0	33
Rat	levo	i v	0.10 $\pm$ 0.01 (LD <sub>50</sub> )	123
	racemic	i v	0.13 $\pm$ 0.02 (LD <sub>50</sub> )	123
	dextro	i v	1.40 $\pm$ 0.14 (LD <sub>50</sub> )	123
	racemic	s c	>2.0	23
	racemic	i v	0.25–0.30	33
Rabbit	racemic	i v	1/2–1/3 epinephrine	247

(108) In the perfused hind limb of the dog, hydroxytyramine is  $\frac{1}{8}$  (108) and in the perfused rabbit ear  $\frac{1}{8}$  (92a) as active as epinephrine

Dilator action on the bronchioles of guinea pigs, dogs and cats is  $\frac{1}{8}$  that of epinephrine (7) Isolated intestinal segments are relaxed by hydroxytyramine when present in concentrations 17 (cat) or 20–40 (rabbit) times that of epinephrine (108) Similarly, the non-pregnant cat uterus is relaxed by doses 80–85 times greater, the uterus *in situ* by doses 100 times greater than the effective dose of epinephrine (108)

### 'EPININE', 1-(3',4'-DIHYDROXYPHENYL)-2-METHYLAMINOETHANE

The pressor potency of 'Epimine' has been reported as  $\frac{1}{4}$  that of racemic epinephrine (25) Comparisons with epinephrine indicate a pressor potency  $\frac{1}{4}$ – $\frac{1}{8}$  in cats (61, 131) and  $\frac{1}{4}$ – $\frac{1}{8}$  in dogs (121, 149, 234) 'Epimine' acts like epinephrine on peripheral blood vessels, causing dilatation in small doses and dilatation followed by constriction with larger doses (197) Constriction of renal vessels has been described (197, 202)

Bronchodilatation in isolated perfused guinea pig lungs in which constriction



was induced by histanune, barium chloride or pilocarpine was found to require 50 times more Epinine than epinephrine (149, 240) Bronchodilatation in dogs, in which constriction was induced by arecoline or histamine, was obtained by doses of 0.4–1.0 mg/kg (42, 195)

Isolated segments of rabbit small intestine (12, 73) and rat colon (92a) are relaxed by 'Epinine' in doses 100 times the effective dose of epinephrine Isolated segments of guinea pig ileum are relaxed by low concentrations (1.4 million) but contracted by higher concentrations (1.400,000) (149) The pupil of both the rabbit and cat is dilated by Epinine at 10 times the effective dose of epinephrine (73) The retractor penis of the dog is contracted by intravenous doses of 10–25 mcg/kg, being somewhat less active than epinephrine (167) The isolated non-gravid uteri of rabbits and guinea pigs are contracted by concentrations of 1.4 million (149)

'KEPHRINE', ADRENALON, 3,4-DIHYDROXYPHENYL METHYLAMINOMETHYL KETONE

'Kephriene' has been reported to be distinctly less pressor than hydroxytyramine (25) Pressor potencies relative to epinephrine are somewhat variable, being reported as 1/200 (132), 1/150 (234), 1/80 (111, 149) Bronchodilatation in isolated perfused guinea pig lungs requires doses approximately 10 times greater than those of epinephrine (149) The isolated guinea pig ileum is relaxed by concentrations 10–20 times greater than those for epinephrine The isolated guinea pig uterus is relaxed whereas the rabbit uterus is contracted by dilutions of 1:1 million (149) Acetylcholine induced activity of the isolated rat uterus is diminished by concentrations 25 times greater than equactive concentrations of epinephrine (92a)

Acute intraperitoneal toxicity in mice is distinctly lower than that of epinephrine An LD<sub>50</sub> value of  $902 \pm 25$  mg/kg, or 196 times that of epinephrine, has been reported (149) The toxic dose intravenously in rabbits is 30 mg/kg, subcutaneously in mice, 2000 mg/kg (33)

'COBEFRIN', CORBASIL, 1-(3',4'-DIHYDROXYPHENYL)-2-AMINOPROPANOL

The pressor potency of 'Cobefrin' in dogs following intravenous injection has been reported to be one-half that of epinephrine (213) Similarly, intravenous injection into cats gave a pressor potency of 0.3–0.4 that of epinephrine (238, 241) With intravenous infusion in dogs, pressor potency was found to be  $\frac{1}{3}$  that of epinephrine (213) Both epinephrine and arterenol are reported to be more vasoconstrictor than 'Cobefrin' when these drugs are injected intraarterially (4)

The heart is strongly stimulated by 'Cobefrin' and responds to effective doses by an increased output, diminished diastolic and systolic volumes, decreased stroke amplitude and increased rate By comparison with epinephrine, it is 4.3 times more stimulating (62) It is also less effective than epinephrine and dioxyephedrine in increasing coronary flow in the isolated perfused rabbit heart (4)

The bronchodilator action of 'Cobefrin' in the isolated perfused guinea pig lungs is  $\frac{1}{15}$  that of epinephrine Histamine bronchoconstriction in anesthetized dogs is antagonized by 'Cobefrin' in doses of 1–3 mg/kg (42) Isolated segments of the

small intestine of the rabbit are relaxed by 2-5 times the effective concentration of epinephrine (73, 215) The effective concentration for relaxation of the isolated rat uterus is 10 times that of epinephrine (92a)

The levo-isomer has been reported to be 2-3 times more effective than epinephrine on the intestine and 200 times more potent than its dextro-isomer (79) Both epinephrine and arterenol are reported to be more stimulating on the ureter, dilator pupillae and nictitating membrane (4) No stimulating effect on the central nervous system of rats was observed with subcutaneous injections of 0.25-5.00 mg/kg. (219)

#### DIOXY-EPHEDRINE, 1-(3',4'-DIHYDROXYPHENYL)-2-METHYLAMINOPROPANOL

The blood pressure response to dioxo-ephedrine is somewhat variable. Some investigators have found this drug to be distinctly pressor, with a dioxo-ephedrine:epinephrine dosage ratio of 41-140 (61, 238, 240). A fall in pressure follows a transient rise and the magnitude of both these responses varies with the dose, the depressor response being prominent with small doses (0.5-100 mcg/kg) and diminishing as the dose is increased (61, 215). Cardiac stimulation is less than with epinephrine, the potency ratio being 1:0.15. However, a better amplitude contraction is maintained (62). Dioxo-ephedrine induces peripheral vasoconstriction and diminishes the cardiac output of cats (188). In the perfused hind leg of the cat, an epinephrine vasoconstrictor ratio of 31.4 has been obtained (187).

The isolated rabbit uterus is stimulated but this action requires a drug concentration 50 times greater than epinephrine (215). The isolated guinea pig intestine is relaxed by doses 5 times larger than the effective dose of epinephrine (215). Bronchodilatation of the isolated perfused guinea pig lung requires doses 14.7 times greater than the effective dose of epinephrine (240). Histamine-induced bronchoconstriction in dogs was found to be effectively relaxed by intravenous doses of 1-2 mg/kg and dioxo-ephedrine was therefore rated as an excellent bronchodilator (42).

No central nervous system stimulation in rats was observed after subcutaneous injections of 1-40 mg/kg (219).

The minimum lethal dose intravenously in rabbits is about 1.0 mg/kg, this produces marked pulmonary edema (215). Subcutaneous doses of 40 mg/kg in rats caused only a 10 per cent mortality (219).

#### 'BUTANEFRINE', ETHYLNORSUPRARENIN, 1-(3',4'-DIHYDROXYPHENYL)-2-AMINO-1-BUTANOL

Butanefrine is predominantly a depressor drug. Intravenous doses of 0.1-1.0 mg/kg cause a sharp fall of blood pressure in anesthetized cats which lasts about 9 minutes (238). Pressor action becomes apparent only after repeated intravenous injections, the depressor response is then replaced by a biphasic action which after further doses is replaced by a purely pressor response (41, 43). In the cat leg perfused with Locke solution, when the doses of 'Butanefrin' and epinephrine were matched quantitatively by repeated injections, 'Butanefrine' was reported to have a mean constrictor potency  $1/1273 \pm 190$  that of epinephrine.

Small doses of the drug were found to be without effect. When defibrinated blood was substituted for Locke's solution, the potency for constrictor action was  $1/238 \pm 15.3$  (43, 187). This suggests that the presence of small amounts of epinephrine (or sympathin) is important for this vasoconstriction. Recent experiments have shown that small amounts of epinephrine or adrenochrome will restore vasoconstriction in perfused rabbit ears when the constrictor response has been exhausted by sympathetic nerve stimulation (70). Excised hepatic veins are constricted by 'Butanefrine', the epinephrine ratio being about 50. Simultaneously with the fall of blood pressure, after intravenous administration, there is an increase in limb and intestinal volumes, a rise in portal and venous pressures and a constriction of the liver. Pooling of blood in the splanchnic bed has been suggested as the principal cause of the fall in blood pressure (41).

The cardiac action of 'Butanefrine' in the Starling heart-lung preparation was less than that of epinephrine. The epinephrine ratio for cardiac output was  $5.3 \pm 1.2$ , for systolic volume,  $6.2 \pm 1.9$  (41).

'Butanefrine' is an effective bronchodilator drug but in the excised perfused guinea pig lungs the doses required are about 71 times greater than those of epinephrine (240). Histamine bronchoconstriction in anesthetized dogs is readily diminished or abolished by the intravenous injection of 1.0 mg/kg of 'Butanefrine'. Inhibitory action on the isolated rat colon is approximately twice greater than that of epinephrine (92a).

The intravenous, intramuscular or subcutaneous administration of 0.5–2.0 mg/kg was found effective in relieving bronchial spasm in asthmatic patients. These doses cause a distinct decrease in diastolic without a significant change in systolic pressure. The pulse rate is moderately increased. Subjective side-effects such as precordial pain, nausea, vomiting and excitation are reported to be less than with epinephrine (239).

Acute intravenous toxicity ( $LD_{50}$ ) in mice was found to be  $117 \pm 1.04$  mg/kg for 'Butanefrine' and  $0.98 \pm 0.184$  mg/kg for epinephrine (239).

**'ISUPREL', 'ALEUDRINE', 1-(3',4'-DIHYDROXYPHENYL)-2-ISOPROPYL-AMINOETHANOL**

'Isuprel', like 'Butanefrine', is a potent vasodepressor drug. The inhibitory actions characteristic of epinephrine have been enhanced by the replacement of the methyl group on the nitrogen by an isopropyl group. Intravenous injection causes a marked fall of blood pressure in anesthetized or decapitated cats, dogs and rabbits (4, 149, 150, 179). Intravenous injection of 0.6–1.0 mcg/kg into anesthetized dogs causes a 38–46 mm Hg fall in blood pressure lasting 3–17 minutes (149, 150). Intramuscular or intra-intestinal injection of 0.10–0.25 mg/kg causes distinct falls in pressure lasting more than 200 minutes. Similar results are obtained in unanesthetized dogs after oral administration (150). Intra-arterial injection of 'Isuprel' causes a reduced peripheral resistance in the renal, mesenteric and femoral vascular beds (4). Subcutaneous administration of 0.15 to 1.0 mgm. to man causes a marked increase in pulse pressure, due in part to a reduction in diastolic pressure. Occasionally, systolic pressure may

increase slightly, this appears to result from increased cardiac action. Similar results have been obtained after sublingual administration (179) 'Isuprel', in these doses, is a potent vasodilator drug (190, 229)

The dog heart-lung preparation reveals a marked cardiac stimulation as evidenced by an increase in stroke and minute volume, a reduction in right auricular pressure and a marked increase in rate (143). The isolated perfused frog, cat and rabbit hearts are stimulated and there is a distinct increase in both rate and amplitude (149, 150, 179). The isolated frog and cat heart give positive inotropic and chronotropic responses to 'Isuprel' and this drug is 10 times more potent than epinephrine in inducing these changes (161). An analysis of the effect on the heart reveals a marked increase in stroke volume and work per beat. As with epinephrine, there was a distinct reduction in the duration of systole (204). Myocardiograms of the dog heart *in situ* (149) and pulse rates in anesthetized and unanesthetized dogs reveal marked tachycardia after intravenous doses of 10 mcg/kg and after intramuscular or oral doses of 0.1-0.2 mg/kg (149, 150). Cardiac inhibition in man, induced by the application of pressure over the carotid sinus, can be prevented by prior medication with 'Isuprel'. The increased sympathetic tone induced by this drug is dominant and therefore prevents the appearance of vagal effects. This stimulant action appears to be largely on the sinus node, lower auricular foci or auriculo-ventricular node and seldom on lower ventricular foci. By contrast, epinephrine and arterenol were reported frequently to induce foci from lower ventricular centers (190).

Histamine bronchoconstriction in the isolated perfused guinea pig lung preparation is readily relaxed by 'Isuprel'. In this action, it seems to be somewhat more effective than epinephrine (179, 225). The bronchoconstriction induced by horse serum in sensitized guinea pigs is abolished (225) and pilocarpine-induced bronchoconstriction in anesthetized dogs is readily antagonized (142). In the latter preparation, it is about 10 times more potent than epinephrine. 'Isuprel' is about 15 times more potent than epinephrine in protecting unanesthetized guinea pigs from histamine mists (225).

'Isuprel' has been reported to be an effective bronchodilator drug in man and to be useful in the treatment of bronchial asthma. It is effective when administered intravenously (0.5-1.0 mg), subcutaneously (1.0 mg), by inhalation (1.0 per cent solution) or orally (10-12.5 mg). These doses cause some tachycardia and palpitation, a small increase in systolic and usually a decrease in diastolic pressure (143, 150, 239). Following the inhalation of 0.5-1.0 per cent solutions, there is a marked increase in vital capacity of asthmatic patients and of subjects with bronchoconstriction induced by histamine or 'Mechoyl' (223).

Isolated intestinal segments are relaxed by concentrations equal to or somewhat less than those of epinephrine (4, 28, 143, 149, 150). The rabbit small intestine and colon *in situ* are promptly relaxed by intravenous doses of 0.03-0.05 mg/kg. Isolated uteri of the rabbit and guinea pig, stimulated by histamine or pituitrin, are relaxed by dilutions of 1:40 million (149, 179). The denervated nictitating membrane of the cat is not contracted by doses of more than 100 mcg/kg (143). The dog retractor penis is relaxed by intravenous doses of 1-2 mg/kg (149, 168).

The acute intravenous toxicity ( $LD_{50}$ ) in mice is  $77 \pm 7$  mg/kg, intraperitoneal toxicity  $494 \pm 14$  mg/kg. Subcutaneous injections of 2–20 mg/kg in dogs cause salivation, restlessness, vomiting, cardiac arrhythmias and occasionally death at 15–20 mg/kg. Subacute and chronic toxicity determinations also suggest that this drug has low toxicity (71).

#### GENERAL CONSIDERATIONS INVOLVED IN SYMPATHOMIMETIC DRUG ACTION

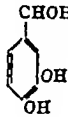
The similarity of the action of epinephrine to sympathetic nerve stimulation (36, 46, 50, 164) has been emphasized previously in this review. Secretion of this hormone by the adrenal gland is probably continuous and it has been determined that cats and dogs secrete about 0.023 mcg/kg/min (228). Large amounts may be secreted in emotional states and this observation has given rise to the theory that epinephrine aids the organism in carrying out the activities associated with pain, fear and anger (45, 47, 49). Recent investigations have demonstrated the presence of *l*-arterenol in extracts of the adrenal medulla (84, 93, 122a, 251). The *l*-isomer has been isolated from commercial epinephrine in pure form (251) and its content estimated as 10–18 per cent of the total (11). These results compare favorably with determinations of the *l*-arterenol content of fresh gland extracts (84). Adrenal medullary tumors have been shown to contain large amounts of *l*-arterenol, this substance accounting for a major portion of the pressor action obtained from extracts of these tumors (93, 122). Experiments in eviscerated spinal cats with the kidneys excluded from the circulation have provided evidence that splanchnic nerve stimulation causes the liberation of both epinephrine and arterenol from the adrenal gland. It was estimated that the arterenol content varied from less than 20 to as much as 80 per cent of the total active material secreted (39).

The possibility that epinephrine, *l*-arterenol or other substances are liberated at sympathetic nerve endings and are the mediators of the nerve impulse has been suggested (13, 50, 72, 165, 193). Stimulation of the sympathetic nerves to the heart, liver, intestine, uterus, pilomotor muscles and other organs liberates a humoral sympathomimetic substance (or substances) which has an effect on the denervated heart, nictitating membrane, intestine, iris and other indicator organs similar to that of epinephrine (34, 46, 48, 52, 53, 56, 136, 206, 210). This possibility was strengthened by the observation that the response to the liberated substance was increased by denervation or by cocainization. Concentrates of solutions obtained by perfusion of stimulated organs were found to have an ultraviolet absorption spectrum quite similar to that of epinephrine (16, 17), were rendered inactive upon exposure to air or heat (155), gave the Viale color reaction (13) and a green fluorescence when exposed to strong alkali. These data suggest that epinephrine is liberated by nerve stimulation. However, certain observations are not wholly in accord with the assumption that epinephrine is the only substance liberated. The pressor response to arterenol in ergotaminized decapitate cats resembles the response to hepatic nerve stimulation more than that to epinephrine (227). An analysis of the physiological responses to epinephrine, arterenol and liver sympathin also supports the assumption that hepatic nerve

stimulation releases a substance which resembles arterenol (101, 196). Subsequently, it was shown that these arterenol-like extracts do not give the fluorescence reaction which characterizes epinephrine (92). Extracts of mammalian adrenergic nerves, bovine and mammalian blood, spleen heart and urine have been described as containing arterenol or an arterenol-like substance (15, 85, 122a). Inasmuch as both epinephrine and *l*-arterenol are obtained from the adrenal medulla, it is not improbable that these substances would be obtained from adrenergic nerve fibers and from organs which contain a large number of adrenergic nerve fibers. The chromaffin cells of the adrenal medulla are analogous to the

TABLE 4

*The effect of varying the N-alkyl group on the adrenergic inhibitory potency of sympathomimetic amines*

<div><div><div>CHOHCH<sub>2</sub>NH R</div><div></div></div></div>		RELATIVE ACTIVITY (In terms of multiples of the effective dose of racemic epinephrine)							
Compound No	Structure R	Uterus					Bronchioles		
		Rat			Guinea pig		Guinea pig		
		(168)	(92a)	(148)	(179)	(254)	(168)	(179)	(225)
1*	H	30	75-300**	10	2.5	100	17	5-7	140
2*	CH <sub>3</sub>	1	1	1	1	1	1	1	1
3	C <sub>2</sub> H <sub>5</sub>		0.5-1.0		1			0.3-0.5	1
4	CH(CH <sub>3</sub> ) <sub>2</sub>		0.5-1.0	0.1	1			0.1-0.3	0.7
5	C <sub>3</sub> H <sub>7</sub>				5.0			3-5	
6	C(CH <sub>3</sub> ) <sub>3</sub>				1			0.03-0.12	

\* For compounds Nos 1 and 2, in references 148 and 168, the comparison is on the basis of the *l*-isomers, 179 and 225, racemic compounds used for comparison, 254, racemic No 1 with *l*-isomer of No 2.

\*\* *l*-Arterenol was used in this investigation.

postganglionic sympathetic neurones. As with adrenal medullary cells, stimulation may cause the liberation of both of these substances.

The theory has been advanced that *l*-arterenol is identical with sympathin E and that this substance plays an essential role in the transmission of adrenergic excitatory nerve impulses (101, 227). However, the inhibitory action of *l*-arterenol is quite marked and can be demonstrated easily. Examination of the data shown in Tables 4 and 5 indicates, in an approximate manner, the relative inhibitory and excitatory actions of both epinephrine and arterenol. The guinea pig uterus is inhibited by these drugs when stimulated or when showing spontaneous activity, and excited when in a quiescent state. The inhibitory dose is 2.5 times (table 4) and the excitatory dose 2 times that of epinephrine (table 5). This suggests that arterenol has the same action as epinephrine for this organ but is somewhat less potent. *l*-Arterenol is more effective than epinephrine in causing

relaxation of the isolated rat colon (92a) The vasoconstrictor potency of epinephrine is 1.5–2.5 times greater than *l*-arterenol in perfused rabbit ears (168). Similarly, arterenol and racemic epinephrine were compared as vasoconstrictors in dogs. The drugs were injected intra-arterially and the resultant change in vasomotor resistance determined. Epinephrine was more vasoconstrictor in the renal, equal in the mesenteric and less in the femoral circulation (4). The similarity of the vascular response to these substances is also illustrated by figure 1. It will be noted in this experiment that the differences in response between epinephrine and *l*-arterenol are quantitative rather than qualitative. By com-

TABLE 5

*The effect of varying the N-alkyl group on adrenergic inhibitory and excitatory actions*

$\begin{array}{c} \text{CHOHCH}_2\text{NH R} \\   \\ \text{C}_6\text{H}_4 \\   \\ \text{OH} \end{array}$		RELATIVE ACTIVITY (In terms of multiples of the effective dose of racemic epinephrine)				
Compound No	Structure R	Uterus*		Nictitating Membrane	Blood Pressure*	
		Guinea Pig	Rabbit	Cat	Dog	
		(149)	(149)	(92a)	(149)	(179)†
1	H	E, 2.0	E, 2.0	E, 1°	E, 0.67	1.0
2	CH <sub>3</sub>	E, 1.0	E, 1.0	E, 1	E, 1.0	1.0
3	C <sub>2</sub> H <sub>5</sub>	E, 20	E, 2.0	E, 45	E/I, 3.3/2.7†	E/I, 2.3/2.7†
4	CH(CH <sub>3</sub> ) <sub>2</sub>	I, 0.5	I, 0.5	I	I, 0.35	I, 0.8
5	C <sub>3</sub> H <sub>7</sub>					I, 2.7
6	C(CH <sub>3</sub> ) <sub>3</sub>	I, 20	I, 2.0		I, 0.35	I, 0.7
7	C <sub>4</sub> H <sub>9</sub>	I, 20	I, 20		I, 5.5	

N-dimethyl analog, cat non-gravid uterus, I, 1/50, blood pressure of spinal cat, E, 1/25, anesthetized dog, 1/40 (251a), spinal dogs, 1/30–1/50 (230a)

\* E, excitation or pressor response, I, inhibition or depressor response

† E followed by I. Ratio based on changes observed, whether pressor or depressor

‡ Results estimated from published graph showing responses at various dose levels

\* *l*-Arterenol was used in this investigation

parison, the vascular response to 'Isuprel' is vasodilator only. Equal vasoconstrictor potency was observed with the *l*-isomers of both drugs in the isolated perfused dog lungs (144). The greater pressor potency of *l*-arterenol may result from greater vasoconstriction in some vascular areas, as noted above for the femoral circulation, from greater stimulation of the heart (in which the drug is reported to be almost 2 times more stimulating than epinephrine (62)), or from a combination of these two effects. Arterenol has been reported to be more excitatory than epinephrine to the pregnant cat uterus. Excitatory action is equal or less in all other organs examined (254). Similarly, the inhibitory action of arterenol can be demonstrated with those organs inhibited by epinephrine but it is less than that observed with the latter substance. The observation that adrenolytic drugs

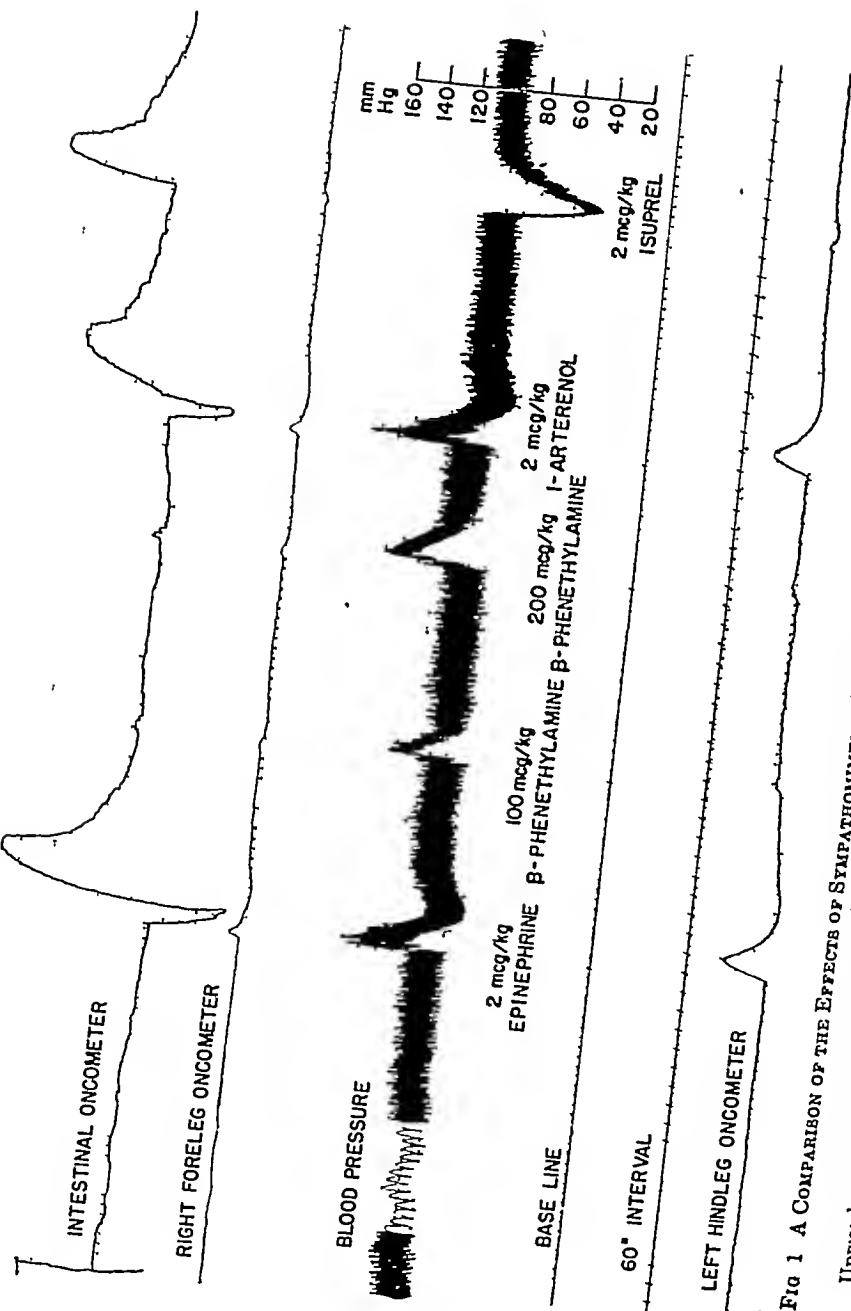


Fig 1 A COMPARISON OF THE EFFECTS OF SYMPATHOMIMETIC AMINES ON BLOOD PRESSURE AND ON LEG AND INTESTINAL VOLUMES, AS DETERMINED BY ONCOMETER

Upward movement in the oncometer record indicates an increase in volume. The axigraph rulings are 5 mm apart.



which readily reverse the pressor action of epinephrine depress but do not reverse the pressor action of arterenol is difficult to understand (86, 185) It is interesting to note that the pressor action of arterenol is reversed in ergotaminized cats However, the dose of arterenol required to produce a vasodepressor response is about 20 times greater than the vasodepressor dose of epinephrine The significance of this observation is uncertain inasmuch as the investigators report that arterenol did not cause vasodilatation in perfused hind limbs of cats treated with Dibenamine (255)

Neither epinephrine nor arterenol is purely excitatory Epinephrine appears to be equal or more active than arterenol as an excitatory agent in most sympathetically innervated organs Arterenol liberation may be important for vasoconstriction but its function in other organs where it is much less potent than epinephrine is not clear Inasmuch as it does not differ greatly from epinephrine as a vasoconstrictor agent, it is difficult to assign to it functions that could not be equally well explained by the release of epinephrine alone However, *l*-arterenol is secreted by the adrenal medulla and is probably released by the stimulation of adrenergic nerves If *l*-arterenol is an intermediate in the synthesis of epinephrine, rapid secretion or an arrest at the terminal stages of synthesis (15) of this hormone might lead to the appearance of some of the intermediate substance or substances in the circulation

It is generally agreed that epinephrine is formed from tyrosine Suggested steps in this synthesis are

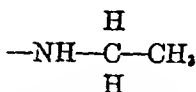
- a Introduction of the second phenolic hydroxyl group (dopa),
- b Decarboxylation (hydroxytyramine),
- c Introduction of the side chain hydroxyl group (arterenol),
- d N-methylation (epinephrine)

It has been suggested that reaction (b) must precede (d) and follow (a), since both tyrosine and N-methyl-dopa apparently are not decarboxylated Perfusion of the suprarenal gland of the cow with N-methyl-dopa does not give rise to epinephrine These data suggest that the primary amine is formed first and that the secondary amine is produced from it by N-methylation (30)

Barger and Dale suggested that the primary amines are imperfect sympathomimetic substances, that their action is mainly excitatory and that they do not possess all the inhibitory effects of epinephrine Investigation of arterenol derivatives in which there are groups larger than methyl substituted on the nitrogen has disclosed that still greater inhibitory potency is obtained with some of these analogs

The rat uterus and guinea pig bronchioles respond to epinephrine and other related sympathomimetic amines by relaxation only and may, therefore, be used as indicators of relative inhibitory potency (table 4) The primary amine (No 1—arterenol) is distinctly less active than the N-methyl analog (No 2—epinephrine) The N-ethyl derivative (No 3) equals or exceeds the N-methyl compound in inhibitory action The greatest potency is obtained with the N-isopropyl (No 4) and N-tertiary butyl (No 6) compounds The N-*n*-propyl (No 5) compound is much less potent than the N-isopropyl derivative and is comparable

to the primary amine as an inhibitory agent. These data suggest that the adrenergic inhibitory receptors respond more readily to compounds in which there is an N-alkyl substitution and in which the substituent is methyl or derivatives obtained by replacing the hydrogens of the methyl group by other methyl groups (Nos 3, 4 and 6). This structural requirement for high inhibitory action was first pointed out with a series of phenolic derivatives (151, 178, 179). The importance of this arrangement is readily seen by comparing compounds No 3-6. No 3 may be considered as



with a single methyl group substituted for one of the hydrogens, and Nos 4 and 6 as having two and three methyl groups, respectively. When the methyl substituent of No 3 is replaced by an ethyl group (No 5), inhibitory potency is greatly reduced.

Interesting results are obtained in organs in which both adrenergic inhibitory and excitatory actions are demonstrable. Representative results are shown in table 5. Derivatives in which the substituent on the nitrogen is ethyl, methyl or hydrogen are excitatory and, except possibly for action on the nictitating membrane and blood pressure, the N-methyl derivative (No 2—epinephrine) is the most potent. Compound No 3 is interesting in that both pressor and depressor actions are obtained. Also, compound No 2 may cause depressor responses under some conditions. If pressor responses alone are considered, we might assume that excitatory action is greatest with the primary amine (No 1) and diminishes progressively with methyl, ethyl, isopropyl and tertiary butyl substitution. However, the pressor response is the result of a complex reaction in which an increased output of the heart and changes in splanchnic, skeletal muscle and skin blood volumes all contribute. As previously pointed out, epinephrine equals or exceeds arterenol as a vasoconstrictor agent when the action is determined by perfusion.

The isolated rabbit or guinea pig uterus provides a comparatively simple indicator of inhibitory and excitatory action. Results obtained with both of these preparations suggest that the N-methyl derivative epinephrine is most excitatory. This action is less with the primary amine and the N-ethyl derivative and is not observed with compounds containing larger N-alkyl groups. The action of these latter substances is predominantly inhibitory. Inasmuch as these uteri respond by both excitation and inhibition to some drugs in this series, it seems probable that both adrenergic inhibitory and excitatory receptors are stimulated by each drug in varying degrees. Motility of the guinea pig uterus may be increased (table 5) or diminished by all of these predominantly excitatory compounds (table 4). As with changes in blood pressure, it may be more accurate to consider these uterine responses as the net result of two opposing actions.

The alcoholic hydroxyl on the side chain is important for both excitatory and inhibitory actions. This is illustrated by the results shown in table 6. Compound No 2 (racemic epinephrine) is twice as potent a uterine stimulant as the corre-

sponding ethane derivative (No 9) The N-methyl ketone derivative (No 14) is about 1/200 as potent as No 2 With histamine-constricted bronchi, No 2 is distinctly more active than either Nos 9 or 14 This indicates the importance of the alcoholic hydroxyl for both excitatory and inhibitory actions Further evidence is obtained by comparing the N-isopropyl derivatives Nos 4, 11 and 16 When the alcoholic hydroxyl is replaced by hydrogen, inhibitory potency is diminished more than 200 times, by oxygen, the compound is inactive

TABLE 6

*The effect of the alcoholic hydroxyl on sympathomimetic potency*

<div style="text-align: center;"> <math display="block">\begin{array}{c} \text{R}' \\   \\ \text{CHCH}_2\text{NH R} \\   \\ \text{C}_6\text{H}_4 \\   \\ \text{OH} \end{array}</math> </div>			<div style="text-align: center;">RELATIVE ACTIVITY (In terms of multiples of the effective dose of racemic epinephrine)</div>				
Comp ound No.	Structure		Uterus* Rabbit (149)	Bronchi oles* guinea pig (149)	Colon rat (92a)	Blood pressure*	
	R	R'				dog (149)	cat (25)
1	H	OH	E, 2 0	I, 140	I, 0 2-1 0**	E, 0 67	E, 0 7
2	CH <sub>3</sub>	OH	E, 1 0	I, 1 0	I, 1 0	E, 1 0	E, 1 0
3	C <sub>2</sub> H <sub>5</sub>	OH	E, 2 0	I, 1 0	I, 1 0	E/I, 3 3/2 7	
4	CH(CH <sub>3</sub> ) <sub>2</sub>	OH	I, 0 5	I, 0 5	I, 1 0	I, 0 35	
5	C <sub>2</sub> H <sub>5</sub>	OH				E/1, 4/2 5 (179)	
6	C(CH <sub>3</sub> ) <sub>3</sub>	OH	I, 2 0	I, 0 5		I, 0 35	
8	H	H				E, 50 (103)	E, 50
9	CH <sub>3</sub>	H	E, 2 0	I, 25	I, 100	E, 6 5	E, 7
10	C <sub>2</sub> H <sub>5</sub>	H					E, 23
11	CH(CH <sub>3</sub> ) <sub>2</sub>	H	I, 200	I, 100		E/I, 620/380	
12	C <sub>2</sub> H <sub>5</sub>	H					E, 140
13	H	O					E, 23
14	CH <sub>3</sub>	O	E, 200	I, 10	I, 4, 20	E, 52	E, 23
15	C <sub>2</sub> H <sub>5</sub>	O					E, 15
16	CH(CH <sub>3</sub> ) <sub>2</sub>	O	Inactive	I, 1000		E/I, 1000/680	
17	C <sub>2</sub> H <sub>5</sub>	O					E, 140

\* E, excitation or pressor response I, inhibition or depressor response

\*\* l-Arterenol was used in this investigation

Results obtained with the cat uterus have shown No 2 to be more inhibitory than No 1 and Nos 9 and 14 more than Nos 8 and 13 (24) The excitatory action of sympathomimetic amines is easily demonstrated with the dog retractor penis preparation (24), although this organ may have adrenergic inhibitory receptors inasmuch as it responds to No 4 by relaxation (168) Compound No 2 is distinctly more excitatory than No 1 (24, 168) The ethane derivatives cause contraction of the retractor penis, the most potent one being No 9 and, in order of diminishing potency, No 10, No 8 and No 12 This order of potency is identical with that found for blood pressure and for the non-pregnant uterus of the cat (24)

These facts are also apparent when the effect on blood pressure is considered. Compound Nos 9 and 14 are much less pressor than No 2. As in the case of the primary amines, there is a marked reduction in pressor potency when the hydroxyl is replaced by hydrogen or oxygen. Compound Nos 3 and 5 are both pressor and depressor, both actions requiring relatively large doses to produce significant changes, the N-isopropyl derivative (No 4) is a strong depressor agent. The corresponding derivatives, Nos 11 and 16, are of very low potency and cause both pressor and depressor responses. Greatest pressor and depressor potency is found with those compounds containing an alcoholic hydroxyl. Among these, maximum excitatory action is obtained with the N-methyl derivative, maximum inhibitory action, with the N-isopropyl or N-t-butyl derivatives.

TABLE 7

*The effect of changes in the structure of the side chain on sympathomimetic potency*

<div style="text-align: center;"> <math display="block">\begin{array}{c} \text{R}' \\   \\ \text{CHOHCHNH R} \\   \\ \text{C}_6\text{H}_4 \\   \\ \text{OH} \end{array}</math> </div>			<div style="text-align: center;"> <b>RELATIVE ACTIVITY</b>  (In terms of multiples of the effective dose of racemic epinephrine) </div>	
Compound No	Structure		Blood pressure*	Bronchioles*
	R	R'		
1	H	H	E, 0.67 (149)	I, 104 (149)
2	CH <sub>3</sub>	H	E, 1.00 (149)	I, 1.0 (149)
4	CH(CH <sub>3</sub> ) <sub>2</sub>	H	I, 0.35 (149)	I, 0.5 (149)
18	H	CH <sub>3</sub>	E, 12 (237), 2-6 (213)	I, 14.9 (240)
19	CH <sub>3</sub>	CH <sub>3</sub>	I/E, 1.0/40-80 (188, 238)	I, 14.7 (240)
20	H	C <sub>2</sub> H <sub>5</sub>	I/E, $\pm$ 50-500/1273† (187)	I, 80 (152)
21	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	I, (203)	
22	CH(CH <sub>3</sub> ) <sub>2</sub>	C <sub>2</sub> H <sub>5</sub>	I, 3 (152)	I, 5.3 (152)

\* E, excitation or pressor response, I, inhibition or depressor response

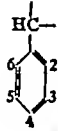
† E value obtained in the isolated perfused cat leg

The size and shape of the group between the benzene ring and the nitrogen as well as of the alkyl substituent of the secondary amine influence excitatory and inhibitory potency. This is illustrated by the data in table 7. The addition of a methyl group to the side chain at R' (No 18) causes a large reduction in pressor potency. The corresponding N-methyl derivative (No 19) is predominantly depressor. Both Nos 18 and 19 are more potent bronchodilators than No 1 but distinctly less active than No 2. An increase in length to an ethyl group in the R' position causes a further reduction in pressor and a simultaneous increase in depressor potency. No 20 has a weak excitatory and a strong inhibitory effect. The N-isopropylbutanol derivative (No 22) is a potent depressor and bronchodilator drug but less active than No 4 in which R' is H. No 20 is a more potent bronchodilator than No 1 but somewhat less potent than No 18. These data suggest that adrenergic excitatory action is diminished by



TABLE 9

The effect of the number and position of the hydroxyls on the benzene ring of sympathomimetic amines

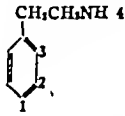
<div></div> A. ETHANOLAMINES						RELATIVE PRESSOR POTENCY*	REFERENCE
Com- pound No	Structure						
	2	3	4	5	6		
2						1	
27	H	H	H	H	H	100-124	61, 236
28	H	OH	H	H	H	10	147
29	H	H	OH	H	H	100	151, 178
30	OH	H	OH	H	H	weak	31
1	H	OH	OH	H	H	0.67-1.0	149, 179
31	OH	H	H	OH	H	weak	163
32	OH	OH	OH	H	H	depressor	163
33	H	OH	OH	OH	H	ineffective	163

B. ETHYLAMINES							
2						1	
34	H	H	H	H	H	183-500	25, 108, 238
35	OH	H	H	H	H	500	25
36	H	OH	H	H	H	70-100	24, 25
37	H	H	OH	H	H	70-105	24, 61, 108, 151
8	H	OH	OH	H	H	50	24, 25, 108
38	OH	OH	OH	H	H	100	25
39	H	OH	OH	OH	H	50	108

\* Expressed in terms of multiples of the effective dose of racemic epinephrine

TABLE 10

The effect of alkoxy substitution on the benzene ring of various sympathomimetic amines

<div>CH<sub>2</sub>CH<sub>2</sub>NH 4</div> <div></div>					RELATIVE PRESSOR POTENCY*	REFERENCES
Compound No	Structure					
	1	2	3	4		
2					1	
40	CH <sub>3</sub> O	CH <sub>3</sub> O	H	H	>400	81
41	CH <sub>3</sub> O	CH <sub>3</sub> O	H	CH <sub>3</sub>	Weak	121
42	CH <sub>3</sub> O	CH <sub>3</sub> O	CH <sub>3</sub> O	H	Ineffective	133
43	CH <sub>3</sub> O	CH <sub>3</sub> O	H	CH <sub>3</sub>	Weak	121
44	H	CH <sub>3</sub> O	CH <sub>3</sub> O	CH <sub>3</sub>	Weak	121
45	CH <sub>3</sub> O	H	CH <sub>3</sub> O	CH <sub>3</sub>	Weak	121

\* Expressed in terms of multiples of the effective dose of racemic epinephrine

that No 28 is much more pressor than No 36. The alcoholic hydroxyl appears to be important for pressor action when the 3-hydroxyl group is present on the benzene ring and relatively unimportant in its absence (Nos 27, 29, 34 and 35) or when there are three hydroxyl groups on the ring (Nos 32, 33, 38 and 39).

The effect of substituting methoxyl for hydroxyl groups on the benzene ring is shown in table 10. In all instances sympathomimetic potency is greatly reduced, the resulting compounds having very little pressor activity. The 2,4-dimethoxyl analog of epinephrine has been reported to be predominantly depressor (238). The replacement of the 3-hydroxyl group on the benzene ring of epinephrine by a 3-amino group diminishes the effects on blood pressure, the non-gravid uterus and the denervated nictitating membrane of anesthetized cats (86a).

#### SUMMARY

The data presented here suggest that, with the exception of the action on the heart, the adrenergic excitatory action of epinephrine equals or exceeds that of other compounds described. It also has inhibitory actions and plays an important role in regulating autonomic activity. *l*-Arterenol appears to be somewhat less potent and its physiological significance less apparent. There is evidence that it is an intermediate in the synthesis of epinephrine. Maximum inhibitory potency is obtained by replacing the hydrogen atoms of the N-methyl group of epinephrine by other methyl groups, to give the corresponding isopropyl or *t*-butyl derivative. Derivatives of this type have not been obtained from biological material and there is no evidence at the present time that they are produced in the animal body. Physiological potency in excess of that obtained with epinephrine suggests the importance of these substances as tools in studying the mechanism of adrenergic inhibitory action. Other modifications in structure, discussed above, diminish both excitatory and inhibitory actions. Of the various modifications, substitution of a methyl or ethyl group on the second carbon of the side chain diminishes inhibitory action less than excitatory action. The structural requirements for adrenergic inhibitory action appear to be less specific than those for excitatory action.

The concept of the sympathins E and I as mediators of adrenergic nerve impulses seems to have outlived its period of usefulness. Various alternative hypotheses have been suggested. The suggestion of two mediators, Sc (contracting substance) and Sr (relaxing substance) proposed more recently (101, 196) seems to be little more desirable than the original concept of the sympathins. The postulation of alpha and beta receptors (4) agrees somewhat better with the experimental data. However, this requires the assumption that stimulation of either receptor may cause either excitation or inhibition, that union with the cell receptor is determined by the structure of the sympathomimetic amine but the nature of the response elicited after union is determined by the organ. Thus, union with the alpha receptors induces contraction of the blood vessels in skeletal muscle and skin, and of the nictitating membrane and uterus, but induces relaxation of intestinal smooth muscle. On the other hand, union with the beta receptors may cause relaxation of blood vessels in skeletal muscle and in the coronary vas-

cular bed, relaxation of the uterus (rat, cat, dog and human) and the bronchioles, and stimulation of the heart

If one assumes excitation or inhibition to result from stimulation of a specific receptor, it is difficult to explain the action of sympathomimetic amines on the heart. Cardiac effects do not correlate well with vascular effects. 'Isuprel' appears to be one of the most potent vasodepressor drugs and at the same time it is more stimulating to the heart than is epinephrine. In order of increasing cardiac stimulation we find No. 5 (N-propyl) < epinephrine < arterenol < No. 3 (N-ethyl) < 'Isuprel'. These observations suggest that a third receptor, specific for sympathetic action on the heart, may be involved, that there may be an adrenergic inhibitory receptor (Ac) involved in the contraction of smooth muscle, an adrenergic inhibitory receptor (Ar) involved in relaxation of smooth muscle, and a third receptor (Ae) concerned in cardiac excitation. The structure of epinephrine could be considered optimal for stimulation of Ac but considerably less than optimal for Ar. The primary amine, arterenol, is almost equal to epinephrine in its effect on Ac but distinctly less effective on Ar. 'Isuprel' or the *t*-butyl analog (No. 6) appears to have the optimum structure for stimulation of Ar. The structural requirement for maximum effect on Ae needs further investigation. The present summary leaves it obvious that these oversimplified hypotheses describe rather than explain the mechanism of adrenergic transmission.

The last two decades have provided us with much detailed information on the factors influencing the activity of sympathetic nerves and their effectors. However, many points remain obscure. The substance or substances involved, the immediate precursor, the manner in which the active agent is liberated, the exact role of the known substances in this sequence of events, the effect of the level of activity of the effector on the resultant response and many other problems require clarification. Progress has been accelerating so that present research efforts can be confidently expected to lead soon to a more complete understanding of these problems. With this increase in knowledge, it is not too much to hope that there will be a correlative development of synthetic agents of great therapeutic importance.

#### REFERENCES

1. ANDERHALDEN, E. AND GELLHORN, E. Das Verhalten des Herzstreifenpräparates (nach Loewe) unter verschiedenen Bedingungen, Das Verhalten des Herzstreifenpräparates (nach Loewe) unter verschiedenen Bedingungen II. Mitteilung. Versuche über den Einfluss von *l*-, *d*- und *d,l*-Adrenalin auf den schlagenden und nichtschlagenden Herzstreifen. Arch. f. d. ges. Physiol., 183: 303-332, 1920, 194: 608-628, 1922.
2. ABELIN, J. Die physiologische Tätigkeit der Harnblase und ihre Beeinflussung durch Produkte der inneren Sekretion und andere wirksame Substanzen. Ztschr. f. Biol., 69: 373-403, 1919.
3. ADLER, L. Beiträge zur Pharmakologie der Beckenorgane. Arch. f. exper. Path. u. Pharmacol., 83: 245-250, 1918.
4. AHLQUIST, R. P. A study of the adrenotropic receptors. Am. J. Physiol., 153: 588-600, 1948.
5. ALLEN, W. J., BANCROFT, H. AND EDHOLM, O. G. On the action of adrenaline on the blood vessels in human skeletal muscle. J. Physiol., 105: 255-267, 1946-47.
6. ALLEN, G. The comparative physiological actions of *dl*- $\beta$ -phenylisopropylamines. I. Pressor effect and toxicity. J. Pharmacol. Exper. Therap., 47: 339-354, 1933.
7. ALLEN, G. AND PRINZMETAL, M. The comparative physiological actions of *dl*- $\beta$ -phenylisopropylamines. II. Bronchial effect. Ibid., 48: 161-174, 1932.
8. AMBERG, S. The toxicity of epinephrin (Adrenalin). Am. J. Physiol., 8: xxxiii-xxxiv, 1903.
9. AMALER, C. AND PICK, E. P. Über die Strophanthinkontraktur der getrennten Kammerhälften des Kaltblutherzens. Arch. f. d. ges. Physiol., 184: 62-78, 1920.



10. ANREP, G. V. AND STACZY, R. S. Comparative effect of various drugs upon the coronary circulation. *J. Physiol.*, 64: 187-192, 1927-28.
11. AUERBACH, M. E. AND ANGELL, E. The determination of arterenol in epinephrine. *Science*, 109: 537, 1942.
12. AUMANN, K. W. AND YOUNG, W. B. Quantitative comparison of responses of isolated and of intact intestine to seven sympathomimetic amines. *Proc. Soc. Exper. Biol. Med.*, 42: 111-112, 1939.
13. BACQ, Z. M. Recherches sur la physiologie du système nerveux autonome, les propriétés biologiques et physico-chimiques de la sympathine comparées à celles de l'adrénaline. *Arch. internat. de physiol.*, 36: 167-246, 1933.
14. BACQ, Z. M. AND BROUHA, L. Recherches sur la physiologie du système nerveux autonome. I. La transmission humorale des excitations nerveuses sympathiques. *Ibid.*, 35: 163-195, 1932.
15. BACQ, Z. M. AND FISCHER, P. Nature de la substance sympathicomimétique extraite des nerfs ou des tissus des mammifères. *Ibid.*, 55: 73-91, 1947.
16. BACQ, Z. M. AND HENRI, V. Preuve Spectrographique de la formation de substances par excitation des nerfs cardiaques. *Compt. rend. Acad. de sc.*, 196: 135-137, 1933.
17. BACQ, Z. M., HENRI, V. AND SCHEPERS, P. Étude spectrographique des substances formées au cours de l'excitation des nerfs cardiaques. *Compt. rend. Soc. de biol. Paris*, 112: 703-704, 1933.
18. BAINBRIDGE, F. A. AND DALE, H. H. The contractile mechanism of the gall bladder and its extrinsic nervous control. *J. Physiol.*, 33: 138-155, 1905-06.
19. BAINBRIDGE, F. A. AND TRIVAN, J. W. Some actions of adrenalin upon the liver. *Ibid.*, 51: 460-468, 1917.
20. BARBOUR, H. G. Die Struktur verschiedener Abschnitte des Arteriensystems in Beziehung auf ihr Verhalten zum Adrenalin. *Arch. f. exper. Path. u. Pharmacol.*, 68: 41-58, 1912.
21. BARBOUR, H. G. AND PRINCE, A. L. The influence of epinephrin upon the coronary circulation of the monkey. *J. Exper. Med.*, 21: 330-337, 1915.
22. BARCROFT, H. A study of the influence of adrenaline on the systemic blood flow. *J. Physiol.*, 76: 339-345, 1932.
23. BARCROFT, H. AND KONZETT, H. Action of noradrenaline and adrenaline on human heart rate. *Lancet*, 256: 147-148, 1949.
24. BARDESS, G. *Organic Chemistry in Biology and Medicine*, 1930. McGraw Hill Book Co., New York.
25. BARDESS, G. AND DALE, H. H. Chemical structure and sympathomimetic action of amines. *J. Physiol.*, 41: 19-59, 1910-11.
26. BATTIELLI, F. AND TARABARDO, P. La toxicité de la substance active des capsules surrénales. *Compt. rend. soc. biol.*, 54: 815-817, 1902.
27. BAVER, W., DALE, H. H., POULASON, L. T. AND RICHARDS, D. W. The control of circulation through the liver. *J. Physiol.*, 74: 343-375, 1932.
28. BEAUVALLÉE, M. Action des amino-alcools diphenoliques: adrénaline, isopropyladrénaline, diéxynéphedrine et diéxynéphedrine sur l'intestin isolé. *Compt. rend. Soc. biol.*, 140: 108-110, 1946.
29. BENCOVITZ, Z. Studies on the visceral sensory nervous system. XII. The response of the isolated esophagus of the frog and the turtle to certain drugs. *Am. J. Physiol.*, 60: 219-233, 1922.
30. BLASCHKO, H. The activity of 3(-)-dopa decarboxylase. *J. Physiol.*, 101: 337-349, 1943.
31. BOUQUET, H. Homologs of adrenaline. *Chem. Ztg.*, 36: 1111, 1914 (*Chem. Abstr.*, 8: 2152, 1914).
32. BOUCHARD, C. AND CLAUDE, H. Recherches expérimentales sur l'adrénaline. *Compt. rend. Acad. de sc.*, 135: 928-931, 1902.
33. BOVET, D. AND BOVET-NITTI, F. *Médicaments du Système Nerveux Végétatif*. Editions S. Karger S. A., Bale, 1948.
34. BRENNEMAN, R. AND VAN DAM, E. Die chemische Übertragbarkeit der Nervenreizwirkung. *Arch. f. d. ges. Physiol.*, 196: 66-82, 1923.
35. BRONIE, T. G. AND CULLIS, W. C. The innervation of the coronary vessels. *J. Physiol.*, 43: 313-324, 1911-12.
36. BRONIE, T. G. AND DIXON, W. E. Contributions to the physiology of the lungs. Part II. On the innervation of the pulmonary blood vessels, and some observations on the action of suprarenal extract. *Ibid.*, 30: 476-502, 1903.
37. BROOKS, C., McPECK, C. AND SKYMOOR, R. J. Action of adrenaline on vasomotors and heart beats studied separately by the artificial control of blood pressure by means of the compensator. *J. Pharmacol. Exper. Therap.*, 11: 165-169, 1918.
38. BÜLBRING, E. AND BURN, J. H. An action of adrenaline on transmission in sympathetic ganglia, which may play a part in shock. *J. Physiol.*, 101: 289-303, 1942.
39. BÜLBRING, E. AND BURN, J. H. Liberation of noradrenaline from adrenal medulla by splanchnic stimulation. *Nature*, 163: 363, 1949.
40. BURTON-OPPEL, R. The vascularity of the liver. XI. The motor reaction in the portal radicles of the liver. *Quart. J. Exper. Physiol.*, 7: 57-74, 1914.
41. CAMERON, W. M., CRIMMON, J. M., WHITSELL, L. J. AND TAINTER, M. L. Analysis of circulatory actions of ethyl norisuprarenin. *J. Pharmacol. Exper. Therap.*, 62: 318-332, 1938.
42. CAMERON, W. M. AND TAINTER, M. L. Comparative actions of sympathomimetic compounds: bronchodilator actions in bronchial spasm induced by histamine. *Ibid.*, 57: 152-169, 1936.
43. CAMERON, W. M., WHITSELL, L. J., CRIMMON, J. M. AND TAINTER, M. L. Further evidences on nature of vasomotor actions of ethylnorisuprarenin. *Ibid.*, 63: 340-351, 1938.
44. CAMPBELL, J. A. The effects of certain animal extracts upon the blood vessels. *Quart. J. Exper. Physiol.*, 4: 1-17, 1911.
45. CANNON, W. B. *Bodily changes in pain, hunger, fear and rage*. 1929. Appleton-Century, New York.
46. CANNON, W. B. AND BACQ, Z. M. Studies on the conditions of activity in endocrine organs. XXVI. A hormone produced by sympathetic action on smooth muscles. *Am. J. Physiol.*, 96: 392-412, 1930-31.

- 47 CANNON, W B AND DE LA PAZ, D : Emotional stimulation of adrenal secretion Ibid , 28: 64-70, 1911
- 48 CANNON, W B AND GRIFFITH, F R Studies on the conditions of the endocrine glands. X. The cardio-accelerator substance produced by hepatic stimulation Ibid , 68: 544-559, 1922
- 49 CANNON, W B AND HOSKINS, R G The effects of asphyxia, hyperpnoea, and sensory stimulation on adrenal secretion Ibid , 29: 274-279, 1911
- 50 CANNON, W B AND LISSAK, K Evidence for adrenaline in adrenergic neurones Ibid , 125: 765-777, 1939
- 51 CANNON, W B AND LYMAN, H The depressor effect of adrenalin on arterial pressure Ibid , 31: 376-393, 1913-13
- 52 CANNON, W B AND ROSENBLUTH, A Studies on conditions of activity in endocrine organs. XXIX. Sympathin E and Sympathin I Ibid , 194: 557-574, 1933
- 53 CANNON, W B AND URIDIL, J E Studies on the conditions of activity in endocrine glands. VIII. Some effects on the denervated heart of stimulating the nerves of the liver Ibid , 58: 353-364, 1931
- 54 CARLSON, A J Studies on the visceral sensory nervous system. XIII. The innervation of the cardia and the lower end of the esophagus in mammals Ibid , 61: 14-41, 1922
- 55 CASTILLO, J C AND DE BRER, E J The tracheal chain. I. A preparation for the study of antispasmodics with particular reference to bronchodilator drugs J Pharmacol Exper Therap , 99: 104-109, 1947
- 56 CATTELL, McK, WOLFF, H Q AND CLARK, D The liberation of adrenergic and cholinergic substances in the submaxillary gland Am J Physiol , 109: 375-385, 1934
- 57 CHANCE, M R A Aggregation as factor influencing toxicity of sympathomimetic amines in mice J Pharmacol. Exper Therap , 87: 214-219, 1948
- 58 CLARK, G A The vaso-dilator action of adrenaline J Physiol , 80: 439-440, 1933
- 59 CLEGHORN, A The action of animal extracts bacterial cultures, and culture filtrates on the mammalian heart muscle Am J Physiol , 2: 273-290, 1898.
- 60 COW, M A Some reactions of surviving arteries J Physiol , 42: 125-143, 1911
- 61 CRIMMON, C A AND TAINTER, M L Comparative pressor efficiency of sympathomimetic amines in the normal state and in decerebrate shock J Pharmacol Exper Therap., 66: 146-170, 1939
- 62 CRIMMON, J M AND TAINTER, M L. Action of sympathomimetic amines on heart-lung preparation: Ibid., 66: 190-203 1938
- 63 CUMMINT, A R The action of optical isomers III Adrenalin J Physiol , 37: 130-138, 1903
- 64 CUMMINT, A R. Further note on adrenalin isomers Ibid , 38: 259-262, 1909
- 65 DAKIN, H D The physiological action of synthetical substances allied to adrenalin Ibid , 32: xxiv xxvi, 1905.
- 66 DALE, H H On some physiological actions of ergot Ibid , 34: 163-205, 1906
- 67 DALE, H H AND RICHARDS, A N The vasodilator action of histamine and of some other substances Ibid , 52: 110-165 1918
- 68 DALEY, I DE B Reactions of the pulmonary and bronchial blood vessels Physiol Rev , 13: 149-184, 1933
- 69 DAUTREAUX, L, PHILIPPOT, E, CHABLIER, R, DUMOULIN, E, AND NOOAKES, F Aerosols medicamenteux. IV Arch int de pharmacodyn et de therap , 63: 117-210, 1942
- 70 DEROGAUX, G AND ROSKAM, J Adrenochrome on sympathetic nerve stimulation J Physiol , 108: 1-8, 1949
- 71 DESTINOSER, B L, BEAVER, D C AND LANDS, A M Toxicity of 1 (3,4 dihydroxyphenyl) 2-isopropylamino-ethanol hydrochloride (Isuprel) Proc Soc. Exper Biol Med , 68: 501-505, 1948
- 72 DIXON, W E AND HAMILL, P The mode of action of specific substances with special reference to secretin J Physiol , 38: 314-338, 1909
- 73 DRAKE, M E JOHN R, RENSHAW, F AND THIESEN, C H The smooth muscle actions of epinephrine substitutes VIII Responses of denervated smooth muscles Arch int pharmacodyn et de therap , 61: 494-503, 1939
- 74 DRINKER, C K AND DRINKER, K R A method for maintaining an artificial circulation through the tibia of the dog, with a demonstration of the vasomotor control of the marrow vessels. Am. J Physiol , 40: 514-521, 1916
- 75 EDMUNDS, C W Some vasomotor reactions of the liver J Pharmacol Exper Therap , 6: 569-590, 1914-16
- 76 EDMUNDS, C W AND ROTH, G B The point of attack of certain drugs acting on the periphery I Action on the bladder Ibid , 15: 189-199 1920
- 77 ELLIOTT, T R On the action of adrenalin (preliminary report) J Physiol , 3f: xx xxi, 1904
- 78 ELLIOTT, T R The action of adrenalin Ibid 32: 401-467, 1905
- 79 ENLILSON B Influence of sympatholytics on action of adrenaline substitutes on isolated intestine, bee venom and ergotamine Acta Physiol Scand , 3: 335-350 1941-42
- 80 EMMERT, J Ueber die Wirkung subkutan eingebrachten Adrenalins Arch f path Anat , 194: 114-121, 1908
- 81 EPSTEIN, D, GUNN, J A AND VIRDEN, C J The action of some amines related to adrenaline I Methoxy phenyl ethylamines J Physiol , 75: 224-248 1932
- 82 ERB, W Experimentelle und histologische Studien über Arterienkrankung nach Adrenalininjektionen Arch f exper Path u Pharmacol 53: 173-212 1905.
- 83 FRANKER, J AND GASSER, H S Studies in secondary traumatic shock III Circulatory failure due to adrenalin Am J Physiol , 49: 345-378, 1919
- 84 EULER, U S V AND HAMBERG, ULLA I nor Adrenaline in the suprarenal medulla Nature 163: 842-843, 1949
- 85 EULER, U S V AND SCHMITZLOW, C O Sympathomimetic activity in extracts of normal human and bovine blood Acta Physiol Scand , 13: 1-8, 1947
- 86 FOLKOW, B, FROST, J AND UYNIS, B Action of adrenaline nor adrenaline and some other sympathomimetic drugs on the muscular, cutaneous and splanchnic vessels of the cat Ibid , 15: 412-420, 1943.

- 86a. FORET, J, CAUWENBERG H VAN AND BACQ Z M Action sympathicomimétique de deux nouvelles amines Comp rend Soc de biol 141 534, 1947
- 87 FRANKLIN K J Actions of adrenaline and acetylcholine on isolated pulmonary vessels and azygos vein of dog J Physiol 75 471-479 1932
- 88 FREEMAN H AND CARMICHAEL, H T A pharmacologic study of the autonomic nervous system in normal men The effects of intravenous injections of epinephrine, atropin, ergotamine and physostigmine upon the blood pressure and pulse rate J Pharmacol Exper Therap, 58: 409-416 1936
- 89 FRÖHLICH, A Eine neue physiologische Eigenschaft des d Suprarenins Vorläufige Mitteilung Zentralbl. f. Physiol., 23 254-256 1909
- 90 FUCHS, R T An initial depression of heart rate in response to epinephrine in human subjects J Pharmacol Exper Therap 63: 143-152, 1939
- 91 FÖHNER, H AND STARLING E H. Experiments on the pulmonary circulation J Physiol, 47: 286-300 1913
92. GAORUM J H AND SCHILD, H A sensitive physical test for adrenaline Ibid., 80: 9-10P, 1934
- 92a. GADNUM, J H, PEART, W S AND VOUT M The estimation of adrenaline and allied substances in blood J Physiol 168 457-481 1949
- 93 GOLDENBERG M, FABER, M, ALSTON, E J AND CHARGAFF, E. C Evidence for the occurrence of nor-epinephrine in the adrenal medulla Science, 109: 534 1949
94. GOLDENBERG M, PINEY, K L BALDWIN, E DE F, GREENE D G AND ROH C E The hemodynamic response of man to nor-epinephrine and epinephrine and its relation to the problem of hypertension Am.-J Med 5 792-805, 1948
95. GOLLA, F L AND STWES, W L. The reversible action of adrenaline and some kindred drugs on the bronchioles J Pharmacol Exper Therap., 5 87-103 1913-14
96. GOTTLIBER R. Ueber die Wirkung des Nebennierenextractes auf Herz und Gefässe Arch f exper Path. u. Pharmacol 43 286-304, 1899
- 97 GRAB, W JANSEN S AND REIM H Ueber die Grösse der Leberdurchblutung. Ztschr Biol 89 324-331, 1929
98. GRANT, R. T AND PEARSON, R. S B Blood circulation in human limb, observations on differences between proximal and distal parts and remarks on regulation of body temperature Clin Sci 3 119-139 1938.
- 99 GRAY, H D SCHÖNBERG, E F AND PASCHOLD, J H Aortic blood flow curves Am J Physiol, 129: 365-367 1940
- 100 GREENE H D, WIGGIA, R. AND BOTER, N H Effects of epinephrine and pitresin on the coronary artery inflow in anesthetized dogs J Pharmacol Exper Therap., 76 378-391, 1942
- 101 GREER, C M PINKSTON, J O BAXTER, J H, JR., AND BRANNON E S. Comparison of response of smooth muscle to arterenol 1-epinephrine and "liver sympathin" I Responses of smooth muscle of iris and of vascular smooth muscle Nor-epinephrine ( $\beta$ -(3,4-dihydroxyphenyl)- $\beta$ -hydroxyethylamine) as a possible mediator in the sympathetic division of the autonomic nervous system Ibid 60 108-109 1937 62 159-227, 1938.
102. GRIMMITH F R, JR., OMACHI A, LOCKWOOD, J E AND LOOMIS, T A Effect of intravenous adrenalin on blood flow, sugar retention, lactate output and respiratory metabolism of peripheral (leg) tissues in anesthetized cat Am. J Physiol., 149: 64-76, 1947
- 103 GRUBER, C M A note on the effect of adrenaline upon strips of excised pregnant human uterus. Endocrin 9: 407-409, 1925
- 104 GRUBER, C M Autonomic innervation of genito-urinary system Physiol Rev, 13: 497-609, 1933
- 105 GRUBER, C M AND ROBERTS, S J XVI. The effect of adrenalin upon the coronary circulation. Am. J Physiol 76 508-529, 1926
106. GUGGENHEIM, M Die biogenen Amine und ihre Bedeutung für die Physiologie und Pathologie des Pflanzlichen und tierischen Stoffwechsels Nordeman Karger
- 107 GUNN, J A. AND GUNN, J W C The action of certain drugs on the uterus of the guinea pig and the rat J Pharmacol. Exper Therap, 5. 527-538, 1913-14
108. GUIN, M R. Physiological action of dihydroxyphenylethylamine and sympatol Quart. J Pharm, 10: 188-211, 1937
- 109 HALL H L. A study of the pulmonary circulation by the trans-illumination method Am J Physiol, 72 446-457, 1925
- 110 HAMILTON, W F AND REMINGTON, J W Some factors in the regulation of the stroke volume Ibid, 153 287-297 1948
- 111 HANDovsky H Ueber die Wirkung des N Methyl, diethylaminocethyl Ephedrins auf Blutdruck und Bronchialwiderstand im Vergleich mit den entsprechenden Wirkungen des Ephedrine Arch int. pharmacodyn et de therap 51: 301-334, 1935
112. HARRIS, F Das schlagend überlebende Herztretfenpräparat Ztschr f d ges exper Med, 6: 301-326 1918
- 113 HASTMAN, F A The differential effects of adrenin on splanchnic and peripheral arteries Am. J Physiol., 33: 435-455, 1915
114. HARTMAN F A., EVANS, J I. AND WALKER, H G The action of epinephrin upon the capillaries and fibers of skeletal muscle Ibid., 85: 91-98, 1928
115. HARTMAN, F A., EVANS, J I. AND WALKER, H G Control of capillaries of skeletal muscle Ibid, 90: 688-688 1929
- 116 HARTUNG W H Epinephrine and related compounds influence of structure on physiological activity Chem Rev, 9: 369-465 1931
- 117 HARTUNG W H Sympathomimetic agents, beta-phenethylamine derivatives Ind and Eng Chem., 37: 126-137 1945.

- 118 HEDBOM, K Ueber die Einwirkung verschiedener Stoffe auf das isolierte Säugetierherz *Skand Arch (Physiol)*, 8: 147-222, 1898
- 119 HEYMAN, C Au sujet de l'influence de l'adrénaline et de l'éphédrine sur les centres vasoregulateurs *Arch. int. de pharmacodyn et de therap*, 35: 307-313, 1928
- 120 HJORT, A M Some physiological properties of certain N methylated- $\beta$ -phenylethylamines *J Pharmacol Exper Therap*, 52: 101-112, 1934
- 121 HJORT, A M, DE BEER, E J, RANDALL, L O Experiences with the biological assay of several sympathicotonic substances including epinephrine *Ibid*, 71: 105-113, 1941
- 122 HOLTON, P Noradrenaline in adrenal medullary tumors *Nature*, 163: 217, 1949
- 122a HOLTZ, P, CREDMER, K and KRONBERG, G Über das sympathicomimetische pressorische Prinzip des Harns ("urosympathin") *Arch f exper Path u Pharmacol*, 204: 228-243, 1947
- 123 HOPPE, J O, SEFFELIN, D K and LANDS, A M An investigation of the acute toxicity of the optical isomers of arterenol and epinephrine *J Pharmacol Exper Therap*, 95: 502-505, 1949
- 124 HORWITZ, O Case of large overdose of epinephrine *Am Heart J*, 33: 102-108, 1947
- 125 HOSKINS, R G The atheric effect of epinephrin upon intestine *Am J Physiol*, 29: 363-366, 1911-12
- 126 HOSKINS, R G and GUNNING, R E L Effects of adrenin on the distribution of the blood V Volume changes and venous discharge in the intestine *Ibid*, 43: 399-407, 1917
- 127 HOSKINS, R G, GUNNING, R E L and BERRY, E L The effects on the distribution of the blood I Volume changes and venous discharge in the limb *Ibid*, 41: 513-528, 1916
- 128 HOSKINS, R G and LOVELETTE, C R The adrenal and the pulse rate *J Am Med Assoc*, 63: 316-318, 1914
- 129 HUNT, R Vasodilator reactions I *Am. J Physiol*, 45: 197-230, 1917-18
- 130 ISSEKUTZ, B V, JR Die Wirkung von Gefässmitteln auf den lokalen Stoffwechsel des Muskels *Arch f exper Path u Pharmacol*, 197: 313-331, 1941
- 131 JACKSON, D The peripheral action of certain drugs with special reference to the lungs, The action of certain drugs on the bronchioles *J Pharmacol Exper Therap*, 4: 291, 320, 1913, 5: 479-510, 1913
- 132 JACROB, E Pharmakodynamische Studie über Adrenalin Gefäßverengernde Atem und sekretorische Wirkung, Pharmakodynamische Studie über Adrenalin Sitz der gefäßverengernden Wirkung, und Wirkung von Adrenalin bei Gegenwart verschiedener vasomotorisch wirkender Mittel *Compt rend de la Soc biol*, 85: 432-433, 910-912, 1921
- 133 JANSEN, M  $\beta$  (2,4,6-Trimethoxyphenyl)- $\beta$ -thylamin, ein Isomeres des Mesalins *Rec trav chim*, 50: 291-312, 1931
- 134 JANUSCHKE, H and POLLAK, L Zur Pharmakologie der Bronchia muskulatur (Zugleich ein Beitrag zur Lehre von der Lungenstarre) *Arch f exper Path u Pharmacol*, 66: 235-250, 1911
- 135 JOHNSON, V, HAMILTON, W F, KATZ, L N and WEINSTEIN, W Studies on the dynamics of the pulmonary circulation *Am J Physiol*, 129: 624-634, 1937
- 136 KAHN, R H Über humorale Übertragbarkeit der Herznervenzirkulation *Arch f d ges Physiol*, 214: 483-486, 1926
- 137 KATZ, L N, LINDNER, E, WEINSTEIN, W and ABRAMSON, D I Effects of various drugs on the coronary circulation of the denervated isolated heart of the dog and cat I Observations on epinephrine, acetylcholine, acetyl- $\beta$ -methylcholine, nitroglycerine, sodium nitrate pitressin and histamine *Arch int de pharmacodyn et de therap*, 59: 399-416, 1938
- 138 KATZ, L N and RODBARD, S The integration of the vasomotor responses in the liver with those in other systemic vessels *J Pharmacol Exper Therap*, 67: 407-423, 1939
- 139 KEHRER, E Physiologische und pharmakologische Untersuchungen an den überlebenden und lebenden in deren Genitalen *Arch f Gynäkol*, 81: 160-210, 1907
- 140 KLEB, P Beiträge zur pathologischen Physiologie der Mageninnervation 3 Mitteilung Zur Atropinfrage: *Dtsch Arch f Klin Med*, 133: 265-285, 1920
- 141 KNAUB, H H and CLARK, A J The action of certain drugs and ions on the rat's uterus *J Pharmacol Exper Therap*, 26: 347-358, 1926
- 142 KONZETT, H Neue broncholytisch hochwirksame Körper der Adrenalinreihe *Arch f Path u Pharmacol*, 197: 27-40, 1940
- 143 KONZETT, H Zur Pharmakologie neuer adrenalinverwandter Körper *Ibid*, 197: 41-57, 1940
- 144 KONZETT, H and HERB, C O Vaso- and bronchomotor actions of noradrenaline (arterenol) and of adrenaline in the isolated perfused lungs of the dog *Arch internat pharmacodyn et de therap*, 78: 210-221, 1949
- 145 KOUNTZ, W B Studies on coronary arteries of human heart *J Pharmacol Exper Therap*, 45: 65-76, 1933
- 146 KURODA, M Observations of the effects of drugs on the ileocolic sphincter *Ibid*, 9: 187-195, 1917
- 147 LANDS, A M and GRANT, J I To be published
- 148 LANDS, A M, LUDWIG, F P, GRANT, J I and ANANENKO, E To be published
- 149 LANDS, A M, NASH, V L, DERTINOEN, B L, GRANGER, H R and McCARHTY, H M The pharmacology of compounds structurally related to hydroxytyramine *J Pharmacol Exper Therap*, 92: 369-380, 1948
- 150 LANDS, A M, NASH, V L, McCARHTY, H M, GRANGER, H R and DERTINOEN, B L Pharmacology of N alkyl homologues of epinephrine *Ibid*, 90: 110-119, 1947
- 151 LANDS, A M, RICHARDS, E E, NASH, V L and HOPFER, K Z Pharmacology of vasodepressor compounds structurally related to sympathomimetic amines *Ibid*, 89: 297-305, 1947
- 152 LANDS, A M, SIEGMUND, O H and ANANENKO, E Bronchodilator action of butanol derivatives *Fed Proc*, 8: 312, 1949
- 153 LANZENDORFF, O Über die Innervation der Koronargefäße *Zentralbl f Physiol*, 21: 551-557, 1907
- 154 LANGLEY, J N Observations on the physiological action of the suprarenal bodies *J Physiol*, 27: 237-256, 1901-

145. LANTZ, A. B. Über die Bildung einer adrenalinähnlichen Substanz nach Reizung des Nervus sympathicus Arch Neerland Sciences exact. nat., 12: 433-444, 1928
146. LAUNOT, L. AND MENOY, B. Zahlenmäßige Angaben über r Adrenalin, I Adrenalin und über Adrenalin Compt. rend Soc. biol., 87 1056-1068, 1922.
147. LESAIGNE, J. Toxicité de l'adrénaline en injection intraveineuse pour le chien, Toxicité de l'adrénaline en injection intraveineuse pour le chat, Action générale de l'adrénaline en injection intra-veineuse chez le chien Influence de la dose. Mécanisme de la mort, Action générale de l'adrénaline en injection intra-veineuse chez le chat Phénomènes d'accoutumance du cœur du chat à l'adrénaline Compt. rend Soc. biol., 56: 632-634, 685-686 709-711, 754-758 800-801, 1904
148. LEVY, A. G. Sudden death under light chloroform anesthesia, Ventricular fibrillation caused by stimulation of the cardiac accelerator nerves under chloroform J Physiol., 42: vii-vii, 1911, 44: xvii-xviii 1912.
149. LEWANDOWSKY, M. Wirkung des Nebennierenextraktes auf die glatten Muskeln der Haut Zentralb f. Physiol., 14: 433-435, 1900
150. LIEB, C. C. AND McWHORTER, J. E. Action of drugs on the isolated gall bladder J Pharmacol. Exper. Therap., 7: 83-98 1915
151. LISAK, K., MARTIN, J. AND ANGYAN, A. Mechanism of action of aleurdine (Isopropyladrenaline) Orvosok Lapja Nepegyógyozás, 2: 129-132, 1946 (Chem. Abs., 42 3074, 1948)
152. LOEWI, S. Das schlagend überlebende Herztzreifenpräparat Ztschr f d ges exper Med., 6: 289-300, 1918
153. LOEWI, S. Über cyclische seitenkettenäthylamine Die pharmakologischen Wirkungen einiger neuer Pyrogalloläthylamin-Verbindungen Skand Arch Physiol., 43: 214-243, 1923
154. LOEWI, O. Übertragbarkeit der Herznervwirkung Arch. ges. Physiol., 189: 237-242, 1921
155. LOEWI, O. Ferrier lecture on problems connected with principle of humoral transmission of nervous impulses: Proc Roy Soc London, s. B., 115: 299-316, 1935
156. LUCAS, D. R. Physiological and pharmacological studies of the ureter III Am J Physiol., 22: 245-278 1908
157. LUDUENA, F. P. Acción de algunas aminas simpaticomiméticas sobre el retractor del pene y la presión arterial del perro Rev. soc. argentina biol., 16: 417-431, 1940
158. LUDUENA, F. P., AMANENKO, E., SIGSMUND, O. H. AND MILLER, L. C. Comparative pharmacology of the optical isomers of arterenol J Pharmacol. Exper. Therap., 95 155-170 1949
159. MACHIZLA, J. Studien am isolierten Herztzreifen (Loewe) Ztschr f d ges exper Med., 14: 287-310, 1921
160. MACHT, D. I. The action of drugs on the isolated pulmonary artery J Pharmacol. Exper. Therap., 6: 13-37, 1914-15
161. MACHT, D. I. The pharmacology of the ureter I. Action of epinephrine, ergotoxin and of nicotine Ibid 8 155-168, 1916
162. MACHT, D. I. AND GIU-CHING TING. Study of antispasmodic drugs on the bronchus Ibid, 18 373-398, 1921
163. MACLEOD, J. J. R. AND PEARCE, R. G. The outflow of blood from the liver as affected by variations in the condition of the portal vein and hepatic artery Am. J. Physiol. 35 87-105, 1914
164. MARKWALDER, J. AND STARLING, E. H. A note on some factors which determine the blood flow through the coronary circulation J Physiol. 47: 275-285, 1914
165. MARRAZZI, A. S. Electrical studies on the pharmacology of autonomic synapses II The action of a sympathomimetic drug (epinephrine) on sympathetic ganglia J Pharmacol. Exper. Therap., 65 395-404, 1939
166. MARRAZZI, A. S. Reduction of sympathetic synaptic transmission as index of inhibition at adrenergic junctions in general Science, 104. 6-8, 1946
167. MARRAZZI, A. S. AND MARRAZZI, R. N. Chemical structure and the definition of sympathin E. Science, 106: 520-521 1947
168. MARSH, D. F. AND HERRING, D. A.: The comparative pharmacology of the N-alkyl 1 (p-hydroxyphenyl) 2 aminoethanols Arch. Int. de pharmacodyn. et de therap., 78 482-498 1949
169. MARSH, D. F., PELLETIER, M. H. AND ROSS, C. A. The comparative pharmacology of the N-alkylarterenols J Pharmacology Exper. Therap. 92 108-120 1948.
170. MAUTNER, H. AND PICK, E. P. Über die durch Shockgifte erzeugten Zirkulationsstörungen II. Das Verhalten der überlebenden Leber Biochem. Ztschr., 127: 72-93, 1923
171. MCGINTY, A. P. AND BAKER, L. S. Effect on heart of overdose of epinephrine, report of case Am Heart J 33 102-105 1947
172. MCGUIGAN, H. AND HYATT, E. G. The primary depression and secondary rise in blood pressure caused by epinephrine J Pharmacol. Exper. Therap. 12: 59-69 1918-19
173. MCMICHAEL, J. AND SHARPEY-SCHAFER, E. P. Cardiac output in man by direct Fick method effects of posture, venous pressure change atropine, and adrenaline Brit. Heart J. 6 33-40 1944
174. MEKE, W. J. AND EYSTER, J. A. E. The effect of adrenalin on the heart-rate Am J Physiol. 38 62-66, 1915
175. MELVILLE, K. I. The antisympathomimetic action of dioxane compounds (F883 and F933), with special reference to the vascular responses to arterenol and nerve stimulation J Pharmacol. Exper. Therap., 59 317-337, 1937
176. MEYER, O. B. Über einige Eigenschaften der Gefäßmuskulatur mit besonderer Berücksichtigung der Adrenalinwirkung Ztschr f. Biology 48: 353-397 1906
177. MORTON, M. C. AND TAINTER, M. L. Effects of sympathomimetic amines on perfused blood vessels J Physiol., 98: 283-282 1940
178. MCCOKE, R. Ein Beitrag zur Analyse der Wirkung des Ephedrins und einiger chemisch verwandter Substanzen Arch f exper Path. u. Pharmacol. 165: 230-243, 1932
179. MUNCH, J. C., GATTONS, V. H. AND PRATT, H. J. Chemistry and pharmacology of an analogue of epinephrine J Am Pharm. Assoc., Sc. Ed., 34: 183-186 1941

- 190 NATHANSON, M H AND MILLER, H Effect of isopropylpinephrine on the rhythmic property of the human heart *Proc. Soc. Exper. Biol. Med.*, 70: 633-636, 1949
- 191 NÉU, M Bedeutung des Suprarenins für Geburtshilfe *Arch. f. Gynakol.*, 85: 617-711, 1908
- 192 OGAWA, S Beiträge zur Gefäßwirkung des Adrenalins *Arch. f. exper. Path. u. Pharmacol.*, 67: 69-110, 1912.
- 193 OLIVER, G AND SCHAFER, E A On the physiological action of extract of the suprarenal capsules; The physiological effects of extracts of the suprarenal capsules *J. Physiol.*, 17: ix xiv, 1895, 18: 230-276, 1895
- 194 ORTH, O S, LEINH, M D, MELLISH, C H AND STUTZMAN, J W Action of sympathomimetic amines in cyclopropane, ether and chloroform anesthesia *J. Pharmacol. Exper. Therap.*, 67: 1-16, 1939
- 195 PEDDEN, J R, TAINTER, M L AND CAMERON, W M Comparative actions of sympathomimetic compounds: bronchodilator actions in experimental bronchial spasm of parasympathic origin *Ibid.*, 35: 242-256, 1935
- 196 PINKSTON, J O, GREEN, C M, BRANNON, E S AND BAXTER, J H Comparison of response of smooth muscle to arterenol, 1-epinephrine and liver sympathin I Response of smooth muscle of iris and of vascular smooth muscle, II Response of uterus, small intestine and nictitating membrane *J. Pharmacol. Exper. Therap.*, 66: 108-109, 115-116, 1937
- 197 PITARNRO, P Effetti prodotti da sostanze adrenalinosimili sopra alcuni distretti vascolari *Arch. int. de pharmacodyn. et de therap.*, 47: 464-485, 1934
- 198 FLUMIER, L Action de l'adrenaline sur la circulation cardio-pulmonaire *J. Physiol. et path. gen.*, 6: 635-670, 1904
- 199 PUCCINELLI, E Sopra alcune modificazioni del deflusso venoso femorale per iniezione di adrenalina Considerazioni sulla partecipazione del circolo femorale ad alcuni fenomeni vasomotori *Riv. di Pat. Sper.*, 12: 281-274, 1934
- 200 QUITTACHAL, W Die Inhalationstherapie bei Asthma bronchial und asthmoiden Zuständen bei der chronischen spastischen Bronchitis und Emphysem *Dtsch. Med. Wochenschr.*, 68: 942-944, 1942
- 201 RANDES, H A AND BRADLEY, S E Systemic and renal circulatory changes following administration of adrenal, ephedrine and paredrine to normal man *J. Clin. Inv.*, 22: 687-693, 1943
- 202 RAYMOND HAMET l'adrenaline et son derive ethylamine peuvent provoquer a la fois de l'hypotension et de la vasoconstriction renale *Compt. rend. Soc. biol.*, 115: 812-815, 1934
- 203 RAYMOND HAMET Änderungen der physiologischen Wirkung der 3,4-Dioxyphenyl  $\beta$ -aminobutanol bei Ernatz der Aminogruppe dieser Verbindung durch eine Methylaminogruppe *Compt. rend. ac. sc.*, 242: 690-692, 1935
- 204 REMINGTON, J W, HAMILTON, W F AND AHLQUIST, R P Interrelation between the length of systole, stroke volume and left ventricular work in the dog *Am. J. Physiol.*, 154: 6-15, 1948
- 205 ROSENBLUTH, A AND CANNON, W B Studies on conditions of activity in endocrine organs. XXVI. A hormone produced by sympathetic action on smooth muscle *Ibid.*, 96: 392-412, 1932
- 206 ROSENBLUTH, A AND MORISON, R S A quantitative study of the production of sympathin *Ibid.*, 109: 209-220, 1934
- 207 ROSALE, R AND PASQUAL, W The coronary circulation in the isolated perfused heart *J. Physiol.*, 74: 1-16, 1932.
- 208 ROTUNIM, E Experimentelle Studien über die Eigenschaften überlebender Gefäße unter Anwendung der chemischen Reismethode *Biochem. Ztschr.*, 111: 219-258, 1920
- 209 RUBSAMEN, W AND KLINGERMANN, N R Pharmakologische Untersuchungen an der überlebenden menschlichen Uterus- und Tubenmuskulatur *Ztschr. f. Geburtshilfe u. Gynakol.*, 72: 272-280, 1912
- 210 RYLAND, P La "transmission humorale de l'action des nerfs cardiaques" de Loewi chez le mammifère *Compt. rend. Soc. de biol.*, 96: 1054-1056, 1927
- 211 SCHAFER, E A AND MINRE, B On the contractility and innervation of the spleen *J. Physiol.*, 20: 1-60, 1896.
- 212 SCHAFER, E S AND LIM, R K S The effects of adrenaline on the pulmonary circulation *Quart. J. Exper. Physiol.*, 12: 157-198, 1919
- 213 SCHAUAMANN, O Über Oxy Ephedrine *Arch. f. exper. Path. u. Pharmacol.*, 160: 127-176, 1931
- 214 SCHAUAMANN, O : Pharmakologische Studien am Histaminasthma des Meerschweinchens *Med. u. Chem.*, 4: 229-247, 1942
- 215 SCHAUAMANN, O Zur Pharmakologie der optischen Isomeren des 3,4-Dioxy- $\alpha$ -Ephedrin Ein Beitrag zum Problem Molekulare Asymmetrie, chemische Konstitution und physiologische Wirkung *Ibid.*, 137, 1936
- 216 SCHMIDT, L Untersuchungen über die toxische Adrenalinwirkung am Meerschweinchen *Ztschr. f. d. ges. exper. Med.*, 9: 285-307, 1919
- 217 SCHMIDT, J Beeinflussung von Druck und Stromvolumen in der Pfortader durch die Atmung und durch experimentelle Eingriffe *Arch. ges. Physiol.*, 126: 165-195, 1909
- 218 SCHULER, W AND WIENEMANN, A Über die Adrenalin-synthese im Reagenzglas unter physiologischen Bedingungen *Hoppe Seyler's Ztschr. f. physiol. Chem.*, 233: 235-250, 1935
- 219 SCHULTZ, J W, REIF, E C, BACHER, J A, JR. LAWRENCE, W S AND TAINTER, M L Further study of central stimulation from sympathomimetic amines *J. Pharmacol. Exper. Therap.*, 71: 62-74, 1941
- 220 SCHULTZ, W H Quantitative pharmacological studies Adrenalin and adrenalin like bodies *Bull. 35, Hyg. Lab. U. S. Public Health*, 1909
- 221 SCHULTZ, W H Experimental criticism of recent results in testing adrenalin *J. Pharmacol. Exper. Therap.*, 1: 291-302, 1909-10
- 222 SCHWAB, O Untersuchungen über die Physiologie und Pathologie der Blasenfunktion IV Mitteilug. Zur Pharmakotherapie der Miktionsstörungen *Arch. f. Klin. Chir.*, 110: 286-308, 1918
- 223 SEGAL, M S Facts and fancies in the management of the seriously ill patient with bronchial asthma: *Dis. Chest*, 14: 795-823, 1948
- 224 SEGAL, M S AND BEAKET, J F Management of bronchial asthma, use of 1 (3', 4-dihydroxyphenyl)-2-isopropylaminoethanol *Ann. Allergy*, 5: 317-336, 1947

- 225 SIEGMUND, O H, GRANGER, H R AND LANDS, A M Bronchodilator action of compounds structurally related to epinephrine *J Pharmacol Exper Therap* 90 254-259, 1947
- 226 SMITH, M L The action of the autonomic drugs on the surviving stomach *Am J Physiol*, 46: 232-243, 1918
- 227 STEHLE, R L AND ELLSWORTH, H C Arterenal as a possible sympathetic hormone *J Pharmacol Exper Therap*, 59 114-121 1937
- 228 STEWART, G N Adrenalectomy and the relation of the adrenal bodies to metabolism *Physiol Rev*, 4: 163-190 1924
- 229 STOLTZENBERGER SEIZOL, M Klinische Untersuchungen zur Behandlung des Asthma bronchiale *Klin Wochenschr.*, 19 1306-1310, 1940
- 230 STOLZ, F AND FLACHER F D R P, 157, 300 (Frdl, 7 689), U S Patent No 862, 675 930, 703
- 230a. STUTZMAN, J W AND ORTH O S Studies of the actions of methyl adrenaline *J Pharmacol Exper Therap.*, 69: 1-12 1940
- 231 SWANSON, E E I The action of ephedrine pseudoephedrine and epinephrine on the bronchioles *J Pharmacol Exper Therap*, 36 541-563 1929
- 232 SWANSON E E SCOTT, C C LEE, H M AND CHEN, K K Comparison of pressor action of some optical isomers of sympathomimetic amines *Ibid* 79 329-333 1943
- 233 SWANSON E E AND WEBSTER, R K Action of ephedrine pseudoephedrine and epinephrine on bronchiolar muscle of isolated lung *Ibid*, 35 327-342, 1930
- 234 TAINTER, M L Comparative actions of sympathomimetic compounds catechol derivatives *Ibid*, 40: 43-64, 1930
- 235 TAINTER, M L Comparative actions of sympathomimetic compounds Influence of cocaine and certain compounds upon the actions of a group of sympathomimetic amines *Quart. J Pharmacol.*, 3 534-558, 1930
- 236 TAINTER, M L Pharmacological actions of pheylethanolamine *J Pharmacol Exper Therap*, 36 20-54, 1929
- 237 TAINTER M L Comparative actions of sympathomimetic compounds catechol derivatives and possible mechanisms of the sensitization-desensitization phenomena of cocaine *Arch int. de pharmacodyn et de therap*, 41 365-376 1931
- 238 TAINTER, M L Comparative actions of sympathomimetic compounds phenyl and substituted phenyl derivatives neopheylethyl ring compounds and aliphatic amines *Ibid*, 46 192-232, 1933
- 239 TAINTER, M L CAMERON, W M WHITSELL L J AND HARTMAN, M M Clinical actions of ethylnoradrenalin *J Pharmacol Exper Therap*, 81 269-277, 1944
- 240 TAINTER, M L, PRUDEN, J R. AND JAMES M Comparative actions of sympathomimetic compounds bronchodilator actions in perfused guinea pig lungs *Ibid* 51 371-386, 1934.
- 241 TAINTER, M L AND TROVONSOV A H Influence of vasoconstrictors on the toxicity of procaine anesthetic solutions *J Am Dent Assoc*. 25 966-979, 1938
- 242 TIFFENEAU, M *Thesis Diet Med*, Paris, 1916 Ref cit 33 (page 114)
- 243 TIFFENEAU M *Titres et Travaux Paris* 1922, Ref cit 33 (page 113)
- 244 TIFFENEAU R. AND BEAUVAILLET M Etude des propriétés bronchodilatatrices de diverses amides amines synthétiques *Compt rend Soc biol* 139 944-945, 1945
- 245 TITON, F P Ueber die Funktion der Bronchialmuskeln *Arch. ges Physiol* 155: 77-91 1913
- 246 TRENDLENBURG P Physiologische und Pharmakologische Untersuchungen an der Isolierten Bronchialmuskulatur *Arch exper Path u Pharmacol* 69: 79-107, 1912-13
- 247 TRENDLENBURG P Adrenalin und adrenalinverwandte Substanzen *Handbuch der Experimentellen Pharmakologie*, Heft 2: 1130-1293 1924
- 248 TRENDLENBURG P AND FLEISCHHAGER, K Ueber den Einfluss des Zuckerstiches auf die Adrenalinsekretion der Nebennieren *Zschr f d ges exper Med.*, 1: 369-395, 1913
- 249 TRIBE, E M Vaso-motor nerves in the lungs *J Physiol* 43 154-170, 1914
- 250 TULLAR, B F The resolution of dl arterenal *J Am Chem Soc.*, 70 2067-2069 1948
- 251 TULLAR, B F The separation of l-arterenal from natural U S P epinephrine *Science*, 109: 536 1949
- 251a VELY, W Propriétés Pharmacologiques de la Diméthyl noradrenaline *Arch int pharmacodyn et de therap*, 77 375-382 1948
- 252 WARMANT, H Recherches pharmacologiques sur le muscle bronchique des poumons isolés du cobaye normal et sensibilisés *Arch int. de pharmacodyn et de therap*, 37: 61-85, 1930
- 253 WEORIA R EMMEX H E HERRICK J F AND MANN, F C Simultaneous action of certain drugs on blood pressure and on flow in right and left coronary arteries *Am Heart J* 20: 557-572, 1940
- 254 WEST G B Quantitative studies of adrenaline and noradrenaline *J Physiol* 106: 418-425, 1947
- 255 WEST, G B The vasodpressor action of noradrenaline *Brit J Pharmacol*, 4 63-67, 1949
- 256 WIGGERS C J The action of adrenalin on the pulmonary vessels *J Pharmacol Exper Therap*, 1 341-348, 1909
- 257 WOODS C J Studies in inaccessible internal hemorrhages II The ineffectiveness of adrenalin in pulmonary hemorrhage *Arch Int. Med*, 3: 360-367, 1909
- 258 WOODBURY R A HAMILTON W F AND TORPIN, R The relationship between abdominal uterine and arterial pressures during labor *Am J Physiol* 121 640-649, 1939
- 259 YOUNG, W B HANEY H F AND AUMANN, K W Relation of the groups of the adrenalin molecule to its cardioaccelerator action *Ibid*, 130: 190-195 1940
- 260 YOUNG W B KARSTENS A I AND AUMANN, K W Effect of vagotomy and of sympathetomy on sensitivity of intestinal smooth muscle to adrenalin *Ibid* 137: 87-93 1942.





# THE ENERGY METABOLISM OF THE FAILING HEART AND THE METABOLIC ACTION OF THE CARDIAC GLYCOSIDES<sup>1</sup>

ALBERT WOLLENBERGER

*Department of Pharmacology, Harvard Medical School, Boston, Massachusetts*

## TABLE OF CONTENTS

	<i>Page</i>
I Introduction	311
II The Energy Metabolism of the Failing Heart	313
A Total Energy Liberation and Utilization	313
1 Spontaneous Failure of the Isolated Heart	313
2 Experimental Heart Failure Induced by Pharmacological Means	316
3 Chronic Congestive Heart Failure	318
B Carbohydrate Metabolism	319
C Balance of Energy-Rich Phosphate	321
III The Metabolic Action of the Cardiac Glycosides	325
A Total Energy Liberation and Utilization by the Heart	325
1 Therapeutic Concentrations	325
a Absence of Heart Failure	325
b Spontaneous Failure of the Isolated Heart	326
c Chemically Induced Heart Failure	327
d Heart Failure Refractory to Cardiac Glycosides	328
e Conclusions	328
2 Toxic Concentrations	329
B Total Energy Yielding Metabolism of Non-Beating Heart Muscle and Other Tissues	330
1 Fermentation	331
2 Respiration	332
3 Mechanisms	335
C Intermediary Metabolism	337
1 Carbohydrate Metabolism of the Beating Heart	337
2 Isolated Tissues and Oxidative Enzymes	339
D Energy-Rich Phosphate Content and Transfer	341
References	344

## I INTRODUCTION

Few classes of drugs have been the subject of such extensive and thorough pharmacological investigation as the cardioactive glycosides of digitalis, strophanthus, and other plants. From the vast literature representing more than a century and a half of research (cf. the comprehensive monographs of Straub (227), Lendle (141), and Weese (241)) emerges a fairly complete and accurate picture of the circulatory effects of these drugs. Above all, the specific action on the heart stands out with great clarity. But to the present day only the gross manifestations of altered organ and tissue function are well understood. The underlying mechanism of the cardiac action is still more or less obscure.

The elucidation of this mechanism, *i.e.*, the analysis of the cardiac action in

<sup>1</sup> The preparation of this review and the original work of the reviewer quoted herein were supported by grants from the Life Insurance Medical Research Fund.



ATP but a closely related dinucleotide (S), which is doubtful (151), this would not constitute a fundamental difference. The recovery metabolism in the heart follows essentially the same pattern as in skeletal muscle. Differences are chiefly quantitative and reflect adaptation to specific energy requirements. As a muscle which is uninterruptedly working throughout its lifetime, nearly always at a steady state, not subject to energy demands imposed by sudden great outbursts of activity such as occur in skeletal muscle, and richly supplied with oxygen and nutrients, the heart, particularly in the warm-blooded animal, is a predominantly aerobic organ, powerfully equipped with respiratory enzymes, but possessing a relatively low ability for anaerobic recovery, as shown by its small phosphocreatine reserve and its low glycolytic power. Detailed information on these and other features of cardiac muscle metabolism is contained in several excellent reviews (38, 44, 63, 152, 181, 217a).

## II THE ENERGY METABOLISM OF THE FAILING HEART

### *A Total Energy Liberation and Utilization*

If intermediary metabolic steps and energy transferring mechanisms are left out of consideration, the problem of where the defect underlying a given type of heart failure is located in the above outlined energy cycle in the heart, reduces itself to the following question: Does the defect concern metabolic energy liberation or does it concern the utilization of the liberated energy for work?

1 *Spontaneous Failure of the Isolated Heart*. A systematic study of the changes in the energy transformations of the heart occurring in failure is feasible only in the isolated organ where the variables of circulatory dynamics cannot only be measured, but where they can be controlled.

The question has been examined most carefully in the mammalian heart, usually the dog heart, isolated in the form of the heart-lung or similar preparation. In this arrangement, the heart is well supplied with oxygenated blood and performs work in a physiological manner, *i.e.*, by pumping blood into the periphery. At first the heart works very competently, but then less and less so, as its contractile power inevitably weakens and it gradually goes into a failure which in many respects is comparable to chronic congestive heart failure and which lends itself easily to experimental analysis. The external work done by this preparation can be accurately measured and, knowing the caloric equivalent of the fuel burned, the total energy set free can be estimated from the oxygen consumption. This is a valid procedure since the heart in the warm-blooded animal contracts an oxygen debt only for brief periods, if at all (63, 84).

The usefulness of the heart-lung preparation in the study of cardiac energetics has been questioned on account of its low mechanical efficiency (195). Indeed, values of the order of 3 per cent are quite common. The low efficiency, interpreted by some authors as indicating failure from the outset, has variously been attributed to unfavorable mechanical conditions inherent in the preparation (79), to denervation of the heart (87, 96), and to the absence of metabolic control through known hormones (98) and unknown substances released by the liver (186, 196). In part, however, it is undoubtedly the consequence of low work

terms of changes on the cellular, subcellular and molecular level, is a research problem whose solution demands much wider use of biochemical methods of approach than has hitherto been the case. Past attacks on the problem along this line have centered mainly around three aspects of the action of the cardiac glycosides: (a) the relation of this action to the physiological role and the metabolism of certain inorganic cations, particularly calcium and potassium (for reviews or summaries of the literature, see 10, 141, 189, 241), (b) effects on the physicochemical state of the protoplasm (141, 241), and (c) effects on the energy transformations in the heart, notably the catabolic processes furnishing the energy for the recovery phase of the cardiac cycle. The present review is concerned chiefly with the last-named effects, the cardiac glycoside-ion relation and the physicochemical changes are discussed only in so far as they supplement information gained from studies of the changes in energy metabolism. Certain aspects of the subject have been reviewed by Lendle (141), Weese (241) and Mardones (159).

Perhaps the most striking of all the effects of the cardiac glycosides on the heart is the strengthening of its contractile power, the positive inotropic action. This effect is most convincingly demonstrated in the failing heart or the hypodynamic heart muscle preparation, in which the impaired contractility can be restored to normal. It may be assumed that the cardiac glycosides reverse whatever chemical or physicochemical change is responsible for the impairment of contractility. Hence, information concerning the mechanism of myocardial failure responding to cardiac glycosides, as well as that of heart failure known to be refractory to these drugs, would also provide clues to the mechanism of the positive inotropic action. With this in mind a discussion of the pertinent literature on the metabolism of the failing heart has been included in the review.

In discussing the relation between the metabolic and contractility changes, the heart will be regarded as a machine which converts chemical into mechanical energy. Studies chiefly on skeletal muscle (67, 211, 228) indicate that the contractile protein complex actomyosin is the structural basis of the muscular machine and adenosine triphosphate (ATP) its immediate source of energy. On stimulation the muscle is somehow activated and contracts, releasing energy during these processes which appears as heat and as tension or work. Enzymatic hydrolysis of ATP releases the free energy which, according to one viewpoint (228), recharges the contractile system, enabling it to return to the relaxed state. Resynthesis of ATP, and of phosphocreatine which is believed to function as a reservoir of energy-rich phosphate bonds (150) (for a contrary view, see (58)), is accomplished during recovery, the energy required for these reactions being produced by the degradation of foodstuff through the processes of glycolysis and respiration.

The indications are that these reactions are also the main chemical events in cardiac muscle. Actomyosin obtained from heart muscle is, according to Szent-Györgyi (228), indistinguishable from that extracted from skeletal muscle, though certain quantitative differences in physical properties have been reported (156), even if it should be true that the energy source for the cardiac systole is not

as the causative factor when the oxygen uptake was estimated from the arterio-venous oxygen difference and the coronary flow, the discrepancy of the results was thought at first to be attributable to the difference in analytical methods. The drawbacks of each method in the determination of the oxygen uptake of the heart have been amply stressed by its opponents (86, 98, 121, 234). To obviate criticism and counter-criticism both methods were improved and refined (118, 169, 185, 250), but without yielding results differing from those obtained previously by the respective investigators. Moe and Visscher (169), using the heart-oxygenator preparation, made simultaneous determinations of the oxygen uptake by the spirometer and blood analysis methods. The methods checked satisfactorily and gave values showing failure in this preparation to be associated with the sharp decline in mechanical efficiency. The explanation for the divergent results of Katz *et al.*, using the same type of preparation, must probably be sought in peculiarities of experimental technique other than the analytical method.

Spontaneous failure of the isolated frog and tortoise heart perfused with Ringer fluid containing blood or serum is entirely due to decreased energy utilization, the oxygen consumption remaining relatively high (39, 40, 53, 61). When some of the same preparations are perfused with pure Ringer fluid, the resulting hypodynamic condition is characterized by a parallel decrease in mechanical activity and total oxygen consumption (39, 40). The resting oxygen consumption is not decreased in this condition. Prolonged perfusion of the mammalian heart with a balanced ion solution likewise leads to a simultaneous decline in work and oxygen consumption (197, 198). In this instance the oxygen supply may be a limiting factor.

The chief cause of the hypodynamic condition of the frog heart after long perfusion with Ringer solution is, according to Clark (35), a loss of lipids from the surface of the cell. The claim that loss of myocardial lipids, in the form of phospholipids, is a cause of human congestive heart failure (138) has not been substantiated (51), and neither has a noticeable decrease in the lipid content of the heart been found in experimental myocardial damage (237).

As Clark (35) has shown, the weakened contractility of the frog heart depleted of lipids can be restored to normal by addition of serum or alcoholic extracts of serum, the active principle being probably a lipid, as well as by low concentrations of sodium oleate, the activity of which is believed to be due to the formation of an insoluble calcium soap at the cellular membrane. The restoration of the contractility of the heart by these substances is accompanied by an increase in oxygen consumption to the normal level (39). Clark (38) draws attention to the observation of Meyerhof (165) that the oxygen consumption of minced muscle is greatly increased on addition of the phospholipid, lecithin, or of one of its components, linolenic acid. Lecithin has been shown by Clark (35) and others (38) to strengthen the beat of the frog heart under the same conditions in which oleate and serum are effective. That phospholipids play a role in oxidative processes has long been suspected (221). A recent study (6) suggests that lipids and phospholipids may serve as "cement substances" to hold in close association groups of enzymes that perform together in metabolic cycles.

levels, at which the resting oxygen consumption constitutes a relatively large fraction of the total metabolism, and is not necessarily a reflection of a poor physiological state (84)

The theoretical and experimental basis for the study of the energetics of the failing heart was laid by the work of Starling and collaborators (187, 225). They demonstrated that the mechanical energy set free in the contraction of the heart depends on its diastolic volume,  $v_e$ , the initial length of its fibers. At a given diastolic volume, the failing heart has a smaller capacity for doing work than the "physiological" heart, in order to maintain a constant level of work, it has to increase its diastolic volume. Later, Starling and Visscher (226) presented data showing that when rate, temperature and chemical conditions are held constant, the oxygen consumed by the mammalian heart is determined by its diastolic volume. According to these authors this is also the case in spontaneous failure of the heart-lung heart. As the heart goes into failure and dilates, the same amount of work can be performed only at the cost of greater oxygen consumption, or when the diastolic volume is kept constant, the oxygen consumption remains the same but the work declines. In either case the mechanical efficiency is decreased. The failing heart is still able to liberate energy from food-stuff at a normal rate, but its ability to convert this energy into work has suffered.

These findings and conclusions concerning the mechanism of the spontaneous failure in the isolated mammalian heart have been confirmed in Visscher's laboratory (169, 185, 235) as well as by other workers (45, 86, 95). They have been challenged by Rühl (203, 206) and by Katz and collaborators, (18, 121, 250), who failed to note a loss of efficiency during failure. Since Rühl did not measure or control diastolic volume and work, his results are not as relevant in this connection as those of Katz *et al* (121) who maintain that when the heart is permitted to fail at approximately constant diastolic volume, there is a coincident decrease in work and oxygen consumption, whereas at a constant work level the diastolic volume increases during development of failure without significant changes in oxygen consumption and mechanical efficiency. In these experiments, apparently, the loss of contractility was associated with a reduction in total energy release and not with a reduced ability to utilize the liberated energy for work.

Katz (118) suggests that diastolic volume may not be the sole factor governing the release of energy by the heart. As other authors (38, 84, 123) have pointed out, if the amount of energy released by the heart were independent of the mechanical conditions prevailing during contraction, cardiac muscle would be in a class apart from skeletal muscle, which liberates extra energy for work (Fenn effect) (cf 67). The crucial experiment to decide this question, namely, the measurement of the heat and work output of cardiac muscle under conditions also permitting isometric contraction, such as in the papillary muscle preparation, has not yet been performed.

Since impairment of energy utilization was always observed to be the cause of spontaneous heart failure in those studies in which oxygen uptake was determined with a spirometer, whereas impairment of energy liberation was found

as the causative factor when the oxygen uptake was estimated from the arterio-venous oxygen difference and the coronary flow, the discrepancy of the results was thought at first to be attributable to the difference in analytical methods. The drawbacks of each method in the determination of the oxygen uptake of the heart have been amply stressed by its opponents (86, 98, 121, 234). To obviate criticism and counter-criticism both methods were improved and refined (118, 169, 185, 250), but without yielding results differing from those obtained previously by the respective investigators Moe and Visscher (169), using the heart-oxygenator preparation, made simultaneous determinations of the oxygen uptake by the spirometer and blood analysis methods. The methods checked satisfactorily and gave values showing failure in this preparation to be associated with the sharp decline in mechanical efficiency. The explanation for the divergent results of Katz *et al*, using the same type of preparation, must probably be sought in peculiarities of experimental technic other than the analytical method.

Spontaneous failure of the isolated frog and tortoise heart perfused with Ringer fluid containing blood or serum is entirely due to decreased energy utilization, the oxygen consumption remaining relatively high (39, 40, 53, 61). When some of the same preparations are perfused with pure Ringer fluid, the resulting hypodynamic condition is characterized by a parallel decrease in mechanical activity and total oxygen consumption (39, 40). The resting oxygen consumption is not decreased in this condition. Prolonged perfusion of the mammalian heart with a balanced ion solution likewise leads to a simultaneous decline in work and oxygen consumption (197, 198). In this instance the oxygen supply may be a limiting factor.

The chief cause of the hypodynamic condition of the frog heart after long perfusion with Ringer solution is, according to Clark (35), a loss of lipids from the surface of the cell. The claim that loss of myocardial lipids, in the form of phospholipids, is a cause of human congestive heart failure (138) has not been substantiated (51), and neither has a noticeable decrease in the lipid content of the heart been found in experimental myocardial damage (237).

As Clark (35) has shown, the weakened contractility of the frog heart depleted of lipids can be restored to normal by addition of serum or alcoholic extracts of serum, the active principle being probably a lipid, as well as by low concentrations of sodium oleate, the activity of which is believed to be due to the formation of an insoluble calcium soap at the cellular membrane. The restoration of the contractility of the heart by these substances is accompanied by an increase in oxygen consumption to the normal level (39). Clark (38) draws attention to the observation of Meyerhof (165) that the oxygen consumption of minced muscle is greatly increased on addition of the phospholipid, lecithin, or of one of its components, linolenic acid. Lecithin has been shown by Clark (35) and others (38) to strengthen the beat of the frog heart under the same conditions in which oleate and serum are effective. That phospholipids play a role in oxidative processes has long been suspected (221). A recent study (6) suggests that lipids and phospholipids may serve as "cement substances" to hold in close association groups of enzymes that perform together in metabolic cycles.

2 *Experimental Heart Failure Induced by Pharmacological Means* There is no need here for a discussion of the general nature of heart failure caused by acute anoxia of the myocardium, whether produced by deprivation of oxygen or by specific respiratory inhibitors such as cyanide. The evidence that these types of failure are due to interference with the supply of energy is clear-cut, even in the case of the frog heart which has some ability for anaerobic survival (38). It is noteworthy that both in oxygen lack as well as in cyanide poisoning, the cardiac glycosides are incapable of restoring the contractility of the myocardium (p. 328).

On the other hand, there are a number of agents with a negative inotropic action on the heart which are known to be antagonized by the cardiac glycosides. The study of the changes in energy metabolism which accompany this action have been confined mainly to heart failure caused by narcotics (general anesthetics, hypnotics) and by alterations in the inorganic ionic environment.

The literature on the action of the narcotics on the cold-blooded heart has been reviewed in the monograph of Clark *et al.* (38). It appears that these compounds depress contractility more severely than oxidative metabolism. The relation was studied in detail by Clark and White (39). They found that with increasing concentrations of such narcotics as ethyl urethane and ethyl alcohol the mechanical response declines at a faster rate than the metabolism, i.e., the mechanical efficiency decreases progressively until complete arrest. At this point, the rate of metabolism is about 75 per cent below the value at full activity, a level comparable to that of the normal resting rate. Deducting this residual from the total respiration, the authors found the proportion between the mechanical and the metabolic depression to be constant over the whole concentration range. They concluded, as they did in a subsequent study (40), that the resting respiration of the frog heart differs from the activity respiration in being more resistant to depressant drugs. As Clark and his collaborators (38) emphasize, these results can be explained without the need of postulating two separate respiratory systems by the hypothesis that inhibition of respiration in the heart by narcotics is secondary to inhibition of the contractile process. In other words, in concentrations which depress the contractile power of the frog heart, the narcotics do not interfere specifically with energy liberation.

The effect of narcotics (chloral hydrate, ethyl alcohol) on the metabolism and the function of the isolated mammalian heart perfused with Locke's solution is the same as in the corresponding cold-blooded preparation, namely, a comparatively more pronounced decline in mechanical performance than in oxygen consumption (70, 199). Whether this relation can be explained by the above hypothesis of Clark is not certain, since values for the resting respiration are not given.

A series of studies on heart failure caused by narcotics in the heart-lung preparation of the dog has yielded conflicting results. According to Gremels (95) the rate of oxygen consumption rises markedly in failure caused by barbiturates, although the work simultaneously declines. An increased oxygen consumption was also observed by Fahr and Buehler (65) in hearts poisoned with chloral hydrate.



The results of other investigators are in sharp disagreement with these reports. According to Rühl (204, 206) and Kiese and Garan (123), the oxygen uptake declines regularly in barbiturate-induced failure, and this decline may even be relatively greater than that of the work. Rühl finds this also to be the case in heart failure caused by avertin (204) and histamine (203). However, he is reluctant to regard this as an indication of improved mechanical efficiency since the carbon dioxide output greatly exceeds the oxygen uptake, which suggests to him that lactic acid is being produced. However, according to Visscher's (234) recalculation and reevaluation of the data, Rühl's oxygen consumption figures are too low and his deductions are consequently misleading. Kiese and Garan (123) leave it undecided whether they were dealing with anaerobic energy production or enhanced conversion of chemical energy into mechanical work. Reim (194) and Gollwitzer-Meier and Krüger (86) found that both the oxygen uptake and the work were reduced in moderate and severe barbiturate-induced failure, and similar observations have been made in the intact dog poisoned with chloral hydrate (108).

According to Gollwitzer-Meier and Krüger (86), the decrease in the oxygen uptake of the heart in barbiturate failure occurs in spite of an increase in diastolic volume. With their sensitive recording technic these authors were also able to observe a decrease in oxygen uptake and consequently an improvement of efficiency after small doses of barbiturate which left the circulation unaffected. The efficiency remained improved when mild failure occurred. These phenomena are taken as an indication of a specific inhibitory effect of barbiturates on the oxidative metabolism of the myocardium. In agreement with this conclusion is the observation (256) that low concentrations of pentobarbital and chlorobutanol reduce the work done by the dog heart more than they reduce what is presumed to be its resting respiration. As was pointed out above, a primary effect of narcotics on the oxidative metabolism of the cold-blooded heart has been negated. These substances are depressants of various cellular functions, depending on the state of the tissue and on external conditions such as, for example, temperature, one or the other function may be the first to be affected. In the cold-blooded heart the primary effect of narcotics over a wide range of concentration appears to be an inhibition of the energy utilizing processes in contraction. In the warm-blooded mammalian heart the energy liberating metabolism may be the first function to suffer, but, as a serious stage of myocardial depression is approached, the conversion of chemical into mechanical energy may become impaired to a greater extent.

Reduction of the  $\text{Ca}^{++}/\text{K}^{+}$  ratio in the extracellular fluid has long been known to diminish the contractile power of the heart. Clark and White (39, 40) have shown that lowering the  $\text{Ca}^{++}$  and increasing the  $\text{K}^{+}$  concentration in the perfusion fluid of the isolated frog heart produce a relatively greater depression of the amplitude of contraction than of oxygen uptake. The mechanical efficiency of the heart declines steadily until the contractions cease. At this time the heart resumes at its normal resting rate, and further changes in ion concentration have little or no effect on this residual fraction of the respiration. On the other hand,

there is a linear relation between the activity (total minus resting) respiration and the mechanical response at all concentrations of ions. Like the analogous effects of narcotics, these phenomena were later interpreted by Clark *et al* (38) to mean that the reduction of the respiratory metabolism is merely the consequence of a depression of the contractile process. Eismayer and Quincke's data (62), on the other hand, suggest that lack of calcium may lower the oxygen uptake of the frog heart more than its mechanical response. The implication that the oxidative metabolism is specifically depressed in the absence of calcium is hard to accept not only in view of the contrary results of Clark and White (39, 40), but also because the depression of the contractility of the frog heart due to calcium lack occurs with a speed much faster than the depression caused by simultaneous complete inhibition of respiration and glycolysis by means of asphyxia and iodoacetate (49). Furthermore, the rapidity of the calcium effect is independent of the frequency of beat, i.e., one of the principal factors determining the rate at which chemical energy is utilized, whereas the rapidity of the effects of asphyxia and iodoacetate is proportional to the frequency of beat (38, 49). It has also been shown that reduction and even complete omission of calcium or excess of potassium in the bathing fluid of non-beating mammalian heart muscle has no immediate effect on the rate of respiration (11, 145, 184).

In heart failure produced in the intact dog by intravenous infusion of potassium chloride the oxygen consumption of the heart is unchanged or slightly increased, the mechanical efficiency is markedly lowered (108).

These facts speak strongly against a direct effect of extracellular calcium lack or potassium excess on the energy yielding metabolism of the heart. The interpretation of the potassium effect is complicated by the fact that excess of this ion impairs the conducting mechanism in the heart (38, 265). This does not seem to be the case in calcium lack. There is ample evidence (38) that the electrocardiogram is unaffected even when the heart is almost completely paralyzed due to the absence of calcium. It is of particular significance that the height of the monophasic electrogram is unaltered (17). This has also been shown to be the case in heart failure caused by barbiturates (133). It can be inferred that barbiturates and lack of calcium weaken the heart beat either by direct impairment of the contractile process or by impairment of the processes intervening between the passage of the action potential and the onset of the contractile response. The latter alternative is the more likely one in the case of calcium lack, since the action of calcium on the heart is believed to be located at the surface of the cell (134, 247).

3 *Chronic Congestive Heart Failure* Nothing definite is known about the mechanism underlying this syndrome and information concerning the point at issue is scant. Harrison (107) has postulated—and this is widely accepted—that a decisive factor in the development of the condition is anoxia of the myocardium due to compensatory cardiac hypertrophy. According to his calculations, the thickness of the hypertrophic fibers does not permit adequate diffusion of oxygen into the internal portions. It has also been estimated (238) that the total capillary surface per unit volume of hypertrophic heart muscle is only one

half as large as in normal heart muscle. However, since the measurements of Dock (55) indicate that in cardiac hypertrophy without coronary disease the coronary flow is more than adequate to provide oxygen in excess of the needs of even the thickest fibers, the anoxia theory of congestive heart failure must be regarded with skepticism. Bing and coworkers (12) report that in patients with congestive heart failure due to mitral stenosis or insufficiency and to arteriosclerotic heart disease the rate of oxygen consumption of the left ventricle per unit weight of muscle is slightly elevated and the mechanical efficiency of work is greatly reduced. The increase in the oxygen consumption, however, is negligible compared to the marked left ventricular enlargement. As the authors point out, this finding raises renewed doubts concerning the validity of Starling and Vischer's (226) postulate that when temperature and the chemical environment are held constant the total energy set free in the heart beat is solely a function of the diastolic volume.

### *B Carbohydrate Metabolism*

Spontaneous failure of the heart in the heart-lung preparation has been reported by Gremels (98) to be preceded and accompanied by a reduction in glucose uptake. As is to be expected from the work of Evans, Grande and Hsu (64) the utilization of glucose is improved by administration of insulin. The heart failure is stated to be correlated with a (hypothetical) depletion of glycogen, and insulin is said to prevent this depletion. But actual determinations of the glycogen content of the heart in the heart-lung preparation show that it remains unchanged even after six hours of work (236), by which time failure is usually pronounced. Confirming earlier results of Bayliss, Müller and Starling (7) Gremels (98) finds that insulin ~~lowers the oxygen~~ consumption of the heart. This effect is attributed to potentiation of a metabolic sparing action believed to be exerted by the peripheral vagus trunk, and the finding is stressed that oxygen consumption is lowered also following administration of acetylcholine (96), a change said to be independent of a simultaneous decrease of the heart rate. According to Bayliss *et al* the decrease in cardiac oxygen consumption produced by (impure ?) insulin is secondary to a decrease in cardiac volume and is not related to metabolic changes. Gremels theorizes that the lower rate of glucose utilization before the administration of insulin and the higher rate of oxygen utilization before the administration of acetylcholine constitute a so-called "energetic insufficiency" of the heart, which he thinks is a necessary condition for the development of dynamic failure. This terminology, which gives hypothetical mechanisms the air of having an experimental foundation, has been extended by Hegghin (110, 111) to clinical heart failure characterized by lengthened electrical systole and simultaneously shortened ejection time. Hegghin's original assumption that this "energetic-dynamic" heart failure is associated with a decreased myocardial glycogen content is not supported by his own experimental studies (94, 111, 112), moreover, there does not seem to be a first order correlation between the amount of glycogen in the heart and the electrocardiogram (93).

Because the heart avidly takes up lactic acid from the blood as a source of energy (see 63) it has been suspected that this process may be disturbed in heart failure. The problem has been studied in the mammalian heart-lung preparation where glycolysis in the lungs and the blood provides the heart with a continuous supply of lactate. The uptake (or output) of lactic acid by the heart has been measured from the coronary arterio-venous lactic acid difference and the coronary flow rate as estimated from the coronary sinus outflow. Since it is not known whether the lactic acid content of the coronary sinus blood and of the Thebesian blood are the same under varying conditions—in view of marked differences in oxygen content (169) this may be doubted—and since the partition of the coronary flow between the two venous channels may change during the development of failure, the results obtained must be accepted with reservations.

An increase in the uptake of lactic acid by the heart is stated by Rühl and Rolshoven (202, 205) to occur during spontaneous failure. According to other investigators (89, 162) the lactic acid uptake in this condition decreases progressively until the balance is finally reversed and the metabolite is given off. An increase in the acidity of the coronary sinus blood during the development of the failure has been interpreted in support of this finding (85). Decreased utilization of lactic acid rather than accelerated breakdown of precursors is held to be the reason for the shift in the balance (85, 89). Since the glycogen content remains unchanged (236) this seems to be a plausible explanation. However, the appearance of excess lactic acid in the coronary sinus blood has to be accounted for. Stimulation of glycolysis from glucose is a possibility which cannot be entirely dismissed.

Granted that the spontaneously failing heart does not retain lactic acid because it is unable to oxidize it, the question arises whether it is only the step to pyruvic acid which is blocked. A study of the fate of added pyruvate, which in the normal heart is rapidly removed (20) and oxidized, presumably by way of the tricarboxylic acid cycle (222), would furnish the answer. That the lactic dehydrogenase system in heart muscle may be particularly sensitive to damage is suggested by the finding (176) that in failure of the Langendorff heart caused by depletion of substrates, partial recovery can be effected by administration of pyruvate, but not of lactate. Information on the metabolism of pyruvate in spontaneous heart failure in the heart-lung preparation would be highly desirable also for the reason that the energy sources of the spontaneously failing heart need to be identified. This is a question of particular concern to those who maintain that total energy liberation in this type of failure is undiminished.

Rühl and Rolshoven (202, 205) also claim to have found an increased lactic acid utilization by the heart in barbiturate- and avertin-induced failure, occurring concomitantly with a reduced oxygen consumption and in spite of a postulated stimulation of anaerobic glycolysis (204). The authors had no way of telling what proportion of lactic acid taken up was synthesized to carbohydrate and what was oxidized. Rühl (205) merely states that if all the lactic acid taken up during failure is completely oxidized, 65 per cent of the total oxygen consumption can be accounted for by this reaction. Since the total oxygen consumption is markedly decreased, this would mean that the oxidation of lactic acid and

also of pyruvic acid in the heart is not inhibited by doses of barbiturates and avertin which inhibit the oxidation of other substrates. In support of this contention one could cite the observation (11) which, however, has been questioned (256) that the oxidation of lactate and pyruvate by heart muscle tissue *in vitro* is highly resistant to narcotics such as avertin. Rühl (205) does not specifically attribute the increased uptake of lactic acid to a suppression of the oxidation of other substrates, but ascribes it to a facilitation of its absorption from the capillaries due to dilatation of the coronary vessels.

In mild anoxemia of the mammalian heart-lung or heart-oxygenator preparation lasting even for considerable periods, the function and metabolism of the organ do not seem to become impaired to an appreciably greater extent than occurs with time at a normal oxygen tension. The usage of oxygen (123) and of lactate (16, 207) is maintained at or near the normal levels, and there is no marked depletion of the glycogen stores, provided glucose or lactate are added (16, 207). Sudden failure of the heart occurs when the oxygen tension in the blood is lowered to below 30 to 40 per cent saturation (16, 99, 123), and this failure is associated with exhaustion of the reserves of glycogen (16, 207) and of energy-rich phosphates (p 322). It is not surprising that in severe myocardial anoxia brought about by occlusion of the coronaries the uptake of lactic acid is arrested (115). This is accompanied and perhaps preceded by destruction of diphosphopyridinenucleotide (DPN), the coenzyme of lactic dehydrogenase (91). It remains to be seen whether this also occurs in less severe myocardial anoxia. The possibility has been suggested (24).

### *C. Balance of Energy-Rich Phosphate*

Knowing that the free energy made available in oxido-reductions is used in muscle for mechanical work through the intercession of ATP and, indirectly, phosphocreatine, with which ATP is in rapid equilibrium, the question of what constitutes, from the point of view of energetics, the cause of failure of the heart can be reformulated somewhat differently. Is the heart failure due to impairment of the generation or of the utilization of phosphate bond energy?

The ATP and phosphocreatine content of the heart at any time reflects the balance between the rates of synthesis and breakdown. The interpretation of changes in content has therefore to take into account the likelihood of changes in the rate of both processes. Unfortunately this is made difficult by the paucity of information concerning the effect of variations in frequency of beat, work, diastolic volume, and other cardiodynamic factors on the ATP and phosphocreatine content of the heart. However, there are indications that normally, within fairly wide limits, it is independent of the activity of the heart (245a, 259). Ordinarily, therefore, a decrease in the ATP and phosphocreatine content of a failing heart may be interpreted as an indication of deficient synthesis, although the possibility of wasteful ATP hydrolysis must be taken into consideration, whereas a constancy or an increase in the content of ATP and phosphocreatine can be accepted as evidence of deficient utilization.

In the rapidly beating warm-blooded heart the continued resynthesis of phos-

phosphocreatine and ATP is highly dependent upon an efficient aerobic metabolism. Under anaerobic conditions phosphocreatine disappears rapidly, followed by depletion of ATP, and the heart fails rapidly and may go into contracture (23, 28, 243). In the frog heart deprived of oxygen these changes are much slower and just enough energy-rich phosphate can be formed by glycolysis to maintain the beat at only moderately reduced strength for as long as an hour, provided the perfusion fluid is alkaline enough to neutralize the lactic acid formed (37).

A decrease in the ATP content of the warm-blooded heart has also been noted in thiamine deficiency (30). In this condition, which eventually leads to disturbances of cardiac function, one of the main pathways of metabolic energy production is blocked due to inactivation of pyruvic oxidase through lack of its coenzyme, thiamine diphosphate. The metabolic defect has been demonstrated in heart muscle of thiamine deficient animals (180, 193). Another important metabolic disturbance affecting cardiac function is caused by excess of thyroid hormone. The disturbance is characterized by exhaustion of the cardiac stores of ATP (9, 161) and phosphocreatine (68, 161, 217, 219). Concomitant losses in glycogen content have been attributed to increases in the rate of beat. However, heart rate increases of the magnitude produced by excess thyroid hormone have no effect *per se* on the phosphocreatine and ATP content of the muscle (259). The mechanism of the metabolic derangement in the thyrotoxic heart is not known. In view of the fact that oxygen consumption is elevated and energy utilization certainly not improved, the depletion of the energy reserves would be consistent with the assumption that either the oxidative synthesis of ATP and phosphocreatine is inefficient or that the energy set free on dephosphorylation of ATP is wasted. Both thiamine deficiency and thyrotoxicosis lead to heart failure which, like cardiac failure produced by acute anoxia, is resistant to the action of cardiac glycosides (p. 328).

In the asphyxiated heart, cold- or warm-blooded, the decline in work capacity is roughly proportional to the decline of the phosphocreatine concentration (27, 28, 37, 243), and on admission of oxygen both return to normal (37). The loss of ATP does not ordinarily become serious under these conditions until the phosphocreatine stores are exhausted, for the reason that ATP is resynthesized at the expense of phosphocreatine, and not necessarily because phosphocreatine might be the primary energy donor for the cardiac systole, as some authors (28, 29) contend. On the basis of experiments such as these, the so-called "phosphagen index"—the ratio of the phosphate of phosphocreatine to the inorganic orthophosphate—has been introduced as a chemical measure of the physiological state of the heart (44). Apart from the fact that this index is objectionable on chemical grounds because in muscle phosphocreatine is not in direct equilibrium with inorganic phosphate, the interpretation given has validity only when the work capacity of the muscle is strictly a function of the rate of formation of energy-rich phosphate.

There are situations where this is not the case. Clark, Eggleton and Eggleton (37) found that when the isolated frog heart is arrested or its beat weakened by lack of calcium, excess of potassium, or by a narcotic such as ethyl urethane,

its phosphocreatine content remains undiminished. Hence, these forms of failure are due to inactivation of the energy utilizing mechanisms and not to metabolic exhaustion. It will be recalled that a similar conclusion was reached by Clark (38) on the basis of respiratory data.

In later experiments Clark and Eggleton (36) observed that frog hearts poisoned with iodoacetic acid in the presence of oxygen eventually reached the stage of contracture or rigor with their phosphocreatine stores intact. Arrest could be hastened by reducing the oxygen pressure and yet, in contrast to arrest in complete anaerobiosis, there was no loss of phosphocreatine. These results are doubly interesting because the occurrence of contracture or rigor without depletion of phosphocreatine is unusual and because the depression of cellular function on reducing the oxygen pressure was not due to deficient synthesis of phosphocreatine. Failure of mammalian heart muscle treated aerobically with iodoacetate is disassociated from phosphocreatine depletion insofar as it occurs considerably ahead of the depletion (29). This is believed to indicate that the contractile mechanism is depressed to a greater extent than the metabolism. This hypothesis, however, could not explain the results of Clark and Eggleton (36), since in their experiments the same concentration of iodoacetate which caused contracture within fifteen minutes at low oxygen pressure had no effect for hours in air. Hence, contractility could not have been directly impaired.

Spontaneous failure of the isolated mammalian heart may or may not be associated with "chemical" failure, depending upon experimental conditions. When the heart is perfused with a salt solution the phosphocreatine content is decreased at the onset of failure and continues to decrease as failure progresses (Mügge, 172, Weicker, 243, 244). According to Mügge the ATP concentration remains unchanged throughout, according to Weicker it is diminished, and, as reported earlier by Parnas and Ostern (182), there is also a loss of adenylic acid, probably due to deamination and possibly further breakdown, as is to be expected when synthesis of ATP is deficient. This failure is also accompanied by a decrease in oxygen uptake (197, 198). The danger of anoxia looms large in the mammalian heart perfused with Ringer, particularly during the setting up of the preparation, and other essential cell constituents besides adenylic acid may be destroyed or washed out (24). These dangers are minimized in the heart supplied with blood. Indeed, analyses performed by the present author (254) reveal that the spontaneously failing heart in the heart-lung preparation retains its normal complement of ATP and is even richer in phosphocreatine than the non-failing heart. In this form of heart failure, therefore, it is the utilization and not the generation of phosphate bond energy which is at fault. In the same publication, unchanged ATP and elevated phosphocreatine values are also reported to have been found in heart failure resulting from the administration of various anesthetics and other toxic drugs, in spite of the fact that some of these compounds have a direct depressant action on the oxidative metabolism of heart muscle (86, 256). It has been suggested (119) that the high phosphocreatine and ATP content of the majority of the failing hearts in these experiments may have been the result of a decrease in the work load. However, the data provide a few

examples showing that a spontaneously failing heart may perform a normal amount of work without differing appreciably, with respect to the distribution of the energy-rich phosphates, from a heart doing little work. Furthermore, it has been found (259) that even very wide variations in "volume" work have little effect on the ATP and phosphocreatine levels in the heart of the heart-lung preparation. Variations in "pressure" work, on the other hand, produce changes in the phosphocreatine level in a direction opposite to the changes in arterial pressure. But in order to produce significant effects the pressure changes have to be pronounced, more pronounced, in fact, than those which occurred in spontaneous failure and in the majority of the drug-induced failures in my above mentioned experiments. Therefore, the adequacy of the energy-rich phosphate supply of the heart in spontaneous experimental failure can hardly be attributed to a sparing action of decreased work. The same can probably be said of failure induced by drugs which do not specifically inhibit the oxidative metabolism.

The instability of phosphocreatine and ATP precludes their determination in human autopsy material. Reasoning that the estimation of creatine after death might be a substitute for the estimation of phosphocreatine in life, Herrman and Decherd (113) and Myers and Mangun (173, 175) have amassed an impressive amount of clinical data, showing, in confirmation of earlier reports (15, 41, 42), the creatine content of the heart to be abnormally low in patients who had died of congestive heart failure. The creatine content in the myocardium of animals with experimentally damaged hearts was likewise found to be lower (113). The authors believe that in all probability the heart in congestive failure has an inadequate supply of phosphocreatine.

Little factual evidence exists as yet to back up this contention. The amount of creatine bound to phosphate in the myocardium is merely a minor fraction of the total creatine (22, 48, 190, 254), and the ratio between the two appears to vary widely from heart to heart (254). Hence caution is required in interpreting differences in myocardial creatine in terms of changes in phosphocreatine. The reported decreases in the creatine content of the heart in congestive failure may turn out to have a significance unrelated to hypothetical decreases in phosphocreatine. It is not known at present what other vital role creatine plays in muscle in addition to serving in phosphate transfer. There are indications (48) that the inherent strength of cardiac muscle varies with its creatine and not with its phosphocreatine content.

The studies of the human heart were extended by Mangun and Myers (157) to include determinations of total acid-soluble purines, the larger part of which was presumably present before death in the form of adenosine compounds. The results show a deficiency of the acid-soluble purines in the cardiac ventricles of patients who had died of myocardial failure. Whether this actually means that the ATP content was low before death remains to be verified.

The same authors (158, 173) suggest that, since phosphocreatine and ATP probably exist in the cell largely as potassium salts (174), loss of these compounds might account for the low potassium content found in the myocardium of patients who had died of congestive heart failure (107, 153, 248). However, the low



potassium content of the heart in congestive failure may also have other causes. There are reasons to believe that the amount of potassium inside the muscle fiber is closely correlated to the physical state of myosin (cf 67, 228). In view of evidence indicating that the solubility (60, 171) and electrophoretic pattern (59, 117) of myosin of fatigued muscles differ from those of rested muscles, the possibility should not be overlooked that the state of myosin in the myocardium might be altered in heart failure.

In a review of their work, Myers and Mangun (175) refer briefly to unpublished observations on dogs with aortic insufficiency. No losses in acid-soluble phosphates were seen in the left ventricles during the early stages of cardiac failure, but in the late stages a decrease in the ATP and phosphocreatine content was noted in two dogs. More determinations of this sort are needed before the significance of the clinical data can be properly assessed. Until such time any pronouncement as to the status of ATP and phosphocreatine in the heart in chronic congestive failure will be more or less arbitrary.

### III THE METABOLIC ACTION OF THE CARDIAC GLYCOSIDES

#### A *Total Energy Liberation and Utilization by the Heart*

1 *Therapeutic Concentrations and Absence of Heart Failure* The cardiac glycosides do not appreciably increase the work of the heart in a good physiological condition. It is important to know whether they may, nevertheless, influence its metabolism. Using the isolated frog heart perfused with Ringer's solution, Eismayer and Quincke (62) obtained considerable increases in oxygen uptake and carbon dioxide output with a very low concentration of strophanthin ( $1 \cdot 10^7$ ) which had no influence on the work output. On addition of small amounts of strophanthin to the isolated frog auricle, David (47) noted a similar increase in respiration, which was later followed by a decline to below the normal level. Since the frequency of beat was lowered and the amplitude of contraction not augmented, the stimulation of metabolism can be regarded, as in Eismayer and Quincke's experiments, to represent a primary effect. In earlier experiments on the isolated frog heart, Gottschalk (90) had failed to note any increase in oxygen consumption by strophanthin  $1 \cdot 10^6$  or by higher concentrations which eventually produced toxic effects. Because an increase in oxygen consumption may be obtainable only under favorable experimental conditions, the findings of Eismayer and Quincke and of David probably carry greater weight than the negative results of Gottschalk.

The fact that in the experiments of Eismayer and Quincke and of David the mechanical efficiency declined, due to the rise in oxygen uptake, is perhaps indicative of a dislocation in the normal interplay of the various cellular functions and may therefore be regarded as a toxic effect. There is no way of telling from the data whether the excess energy liberated is completely wasted as heat or whether some energy requiring activity other than contraction-relaxation is stimulated. Since under different circumstances the heart is made to work more economically by the cardiac glycosides, the latter alternative seems more likely.

The argument that the liberation of the extra energy is itself a phenomenon characteristic of the "toxic" as contrasted to the "therapeutic" phase of action of cardiac glycosides on the heart (84, 97) is not very helpful because the processes underlying both phases may be the same

The gaseous metabolism of the non-failing heart-lung preparation of the dog as well as its work and diastolic volume has been found by Gollwitzer-Meier and Krüger (86) to be practically unchanged after administration of therapeutic doses of strophanthin Gremels (97) has presented data showing the oxygen consumption and the frequency of beat of the freshly prepared heart-lung preparation to be markedly reduced following the administration of small amounts ( $5 \mu\text{gm}$ ) of strophanthin or digitoxin These effects seem to be due to sensitization of the denervated heart to residual vagal activity because they can be reproduced by infusion of minute amounts of acetylcholine which in themselves are not effective (cf also 46) Gremels believes that the decrease in oxygen consumption is a phenomenon independent of the decrease in heart rate

Cattell (26) has found that the tension and the total heat produced in the twitch of the frog sartorius suspended in a gaseous environment are both increased after previous exposure to ouabain, the mechanical efficiency remaining unchanged This is soon followed by a decrease in tension, heat output, and efficiency, and finally by loss of excitability The initial changes and the inexcitability, but not the decrease in efficiency, are probably related to the escape of potassium from the muscle fiber and its accumulation at the membrane (103) Increased movement of potassium is a prominent chemical feature of the toxic and perhaps also of the positive inotropic action of the cardiac glycosides on cardiac muscle (see p 343)

b *Spontaneous Failure of the Isolated Heart* In the isolated mammalian heart perfused with Locke solution in which spontaneous failure is characterized by a proportionally equal decline in work and total energy liberation (197, 198), the positive inotropic action of strophanthin is associated with an increase in oxygen uptake, without appreciable changes in mechanical efficiency (199) This is also true in hearts beating isometrically at approximately constant diastolic volume

The changes in cardiac energetics characterizing the spontaneous failure of the heart in the mammalian heart-lung preparation are likewise reversed by the cardiac glycosides Most authors agree that the heart is enabled to perform more work with a relatively or even absolutely smaller expenditure of energy (86, 95, 168, 185) Claims that the oxygen consumption increases in proportion to the work done and that consequently the improvement in work performance is due to increased liberation of energy (206, 208) have been dismissed as based on questionable methods of estimating work (84) and oxygen consumption (234) When the venous blood supply to the heart is kept constant during recovery from spontaneous failure the increase in work is accompanied by an actual decrease in oxygen consumption (86, 95) The decreased oxygen consumption is said to be proportional to a decrease in external diastolic volume (86) While there cannot be the slightest doubt concerning the improvement of energy utiliza-

tion, the complexity of the changes renders their interpretation difficult. The analysis of the relation between the cardiodynamic and energetic changes becomes easier if either work or diastolic volume is kept constant. The latter is the procedure followed by Peters and Visscher (185). At constant external diastolic volume the oxygen consumption of the spontaneously failing heart is found to increase following the administration of various cardiac glycosides (scillaren, ouabain, diglanid). The utilization of liberated chemical energy for work is improved even more. At the peak of the therapeutic effect, just before irregularities occurred, the increases in oxygen consumption amounted to 16 to 52 per cent, the increases in mechanical efficiency, to 60 to 153 per cent. Peters and Visscher conclude that this represents an improvement in "true" myocardial efficiency. The increase in metabolism is probably not merely a borderline manifestation of poisoning, as Gollwitzer-Meier (84) suggests, since Peters and Visscher's curves show it to occur before the maximum therapeutic effect is reached. However, these authors think that it may be due to a change in the hydration of the myocardium which, judging from their discussion of hydration changes in failure, presumably results in increases in the internal and mean diastolic volumes. Moe and Visscher (168) state, though this is by no means apparent from their published data, that there is no change in total energy output following small doses of diglanid (0.04 to 0.1 mg.) which increase the efficiency of the spontaneously failing heart in the heart-lung preparation. In view of these interpretations and findings and the apparent absence of a metabolic effect in the non-failing mammalian heart (86), it is not possible at present to attach decisive significance to the reported increases in energy liberation, although there is some justification for doing so in the case of the frog heart (p. 325).

c *Chemically Induced Heart Failure* In the isolated frog heart weakened by ethyl alcohol or lack of calcium, low concentrations of strophanthin ( $1.2 \times 10^6$ ) produce an increase in respiratory activity which appears to be secondary to the increase in mechanical activity (62). In the mammalian heart-lung preparation a variety of substances with a negative inotropic action have been shown to be antagonized by the cardiac glycosides. Where the energetics of the heart have been studied a reversal of the changes produced by the depressants has usually been seen. Conflicting statements concerning the direction of the reversal stem from disagreement as to the nature of the changes (see pp. 316-317). According to Fahr and Buehler (65) therapeutic doses of diglanid lower the supposedly elevated oxygen uptake of the heart in failure induced by chloral hydrate apparently in proportion to a reduction in diastolic volume. At approximately constant diastolic volume the oxygen uptake remains unaltered. But Peters and Visscher (185) present an example of a dramatic relief by scillaren of heart failure due to ethyl alcohol where at constant diastolic volume the oxygen consumption rose 77 per cent and the mechanical efficiency 204 per cent. Gremels' assertion (95) that the respiration of the heart weakened by barbituric acid derivatives is lowered by cardiac glycosides has been repeatedly denied (86, 206, 208). Although the diastolic volume of the barbiturate-poisoned heart is decreased by strophanthin (86), the oxygen uptake may actually increase (208).

The argument that the liberation of the extra energy is itself a phenomenon characteristic of the "toxic" as contrasted to the "therapeutic" phase of action of cardiac glycosides on the heart (84, 97) is not very helpful because the processes underlying both phases may be the same

The gaseous metabolism of the non-failing heart-lung preparation of the dog as well as its work and diastolic volume has been found by Gollwitzer-Meier and Krüger (86) to be practically unchanged after administration of therapeutic doses of strophanthin Gremels (97) has presented data showing the oxygen consumption and the frequency of beat of the freshly prepared heart-lung preparation to be markedly reduced following the administration of small amounts ( $5\text{ }\mu\text{gm}$ ) of strophanthin or digitoxin These effects seem to be due to sensitization of the denervated heart to residual vagal activity because they can be reproduced by infusion of minute amounts of acetylcholine which in themselves are not effective (cf also 46) Gremels believes that the decrease in oxygen consumption is a phenomenon independent of the decrease in heart rate

Cattell (26) has found that the tension and the total heat produced in the twitch of the frog sartorius suspended in a gaseous environment are both increased after previous exposure to ouabain, the mechanical efficiency remaining unchanged This is soon followed by a decrease in tension, heat output, and efficiency, and finally by loss of excitability The initial changes and the inevitability, but not the decrease in efficiency, are probably related to the escape of potassium from the muscle fiber and its accumulation at the membrane (103) Increased movement of potassium is a prominent chemical feature of the toxic and perhaps also of the positive inotropic action of the cardiac glycosides on cardiac muscle (see p 343)

b *Spontaneous Failure of the Isolated Heart* In the isolated mammalian heart perfused with Locke solution in which spontaneous failure is characterized by a proportionally equal decline in work and total energy liberation (197, 198), the positive inotropic action of strophanthin is associated with an increase in oxygen uptake, without appreciable changes in mechanical efficiency (199) This is also true in hearts beating isometrically at approximately constant diastolic volume

The changes in cardiac energetics characterizing the spontaneous failure of the heart in the mammalian heart-lung preparation are likewise reversed by the cardiac glycosides Most authors agree that the heart is enabled to perform more work with a relatively or even absolutely smaller expenditure of energy (86, 95, 168, 185) Claims that the oxygen consumption increases in proportion to the work done and that consequently the improvement in work performance is due to increased liberation of energy (206, 208) have been dismissed as based on questionable methods of estimating work (84) and oxygen consumption (234) When the venous blood supply to the heart is kept constant during recovery from spontaneous failure the increase in work is accompanied by an actual decrease in oxygen consumption (86, 95) The decreased oxygen consumption is said to be proportional to a decrease in external diastolic volume (86) While there cannot be the slightest doubt concerning the improvement of energy utiliza-

importance in the activity of the tissue is also suggested by the great variety of chemical changes and pharmacological agents which are antagonized by the cardiac glycosides, comprising, in addition to alterations of the extra-cellular ionic environment and the changes responsible for the development of spontaneous heart failure, not only hypnotics, histamine, and camphor, but a host of compounds not used in the above studies, such as volatile anesthetics, cocaine, heavy metals and quinoline derivatives

2 *Toxic Concentrations* Doses of strophanthin leading eventually to ventricular contracture<sup>2</sup> have been reported to reduce the respiration of the isolated ventricle of the frog heart (62, 90) The same effect has been observed in the strophanthin-poisoned frog auricle (47) In all these experiments the decline in respiration paralleled the decline in work or amplitude of contraction and was interpreted as a secondary effect Near the point of contracture at minimal amplitudes of beat, the oxygen uptake, according to Gottschalk (90), is only about 30 per cent of the initial rate, which is a value only slightly higher than the normal resting respiration of the frog ventricle (38) This observation has not been confirmed On the contrary, frog ventricular muscle treated with toxic doses of ouabain and described to be in a state of contracture has repeatedly been found to respire at a rate exceeding by far the resting respiration (233, 262) That Niemeyer and Lira, in Mardones' laboratory (159), could not find a change in the oxygen uptake of the isolated frog heart treated with high concentrations of digilanid is perhaps not surprising since their measurements extended over a period of only ten minutes Salomon and Riesser's (210) data which show no change in the oxygen uptake of isolated frog and mouse hearts in highly toxic solutions of digitoxin and strophanthin are not pertinent to this discussion, because no information on the mechanical activity or the physical condition of the muscle is given

Ventricular contracture due to poisoning with cardiac glycosides rarely occurs in the mammalian heart supplied with blood Instead, foci of discharge are set up in the ventricular musculature which give rise to tachycardia and, in combination with a slowing or block of conduction, to severe irregularities of rhythm and eventually to fibrillation The oxygen uptake of the heart in tachycardia can be expected to be high, but it seems to be elevated even in bradycardia, if there are extrasystoles (168) In the isolated warm-blooded heart perfused with Locke's solution, contracture is the usual endpoint of cardiac glycoside poisoning, and in this state oxygen consumption is markedly augmented (199) This has also been observed in an instance of contracture produced by strophanthin in a mammalian heart-lung preparation (86) No information is available on the metabolism of skeletal muscle in contracture produced by high concen-

<sup>2</sup> This effect of cardiac glycosides, incorrectly (227) called "systolic" standstill, belongs to the group of phenomena covered by Gasser's (78) definition of "contracture" as a sustained, but non-propagated and reversible muscular shortening or tension development The reversibility of the cardiac glycoside contracture has been demonstrated in the frog heart by means of washing with heart muscle extracts (109) and with thiamine triphosphate (188)

This probably depends on the changes in work performance. There may also be an increase in mechanical efficiency (86), however, as in the reversal of heart failure produced by camphor (95), this increase can be accounted for by the resumption of the normal relation between work and oxygen consumption with respect to output, i.e., by the well-known improvement of mechanical efficiency accompanying the rise in volume work of the non-failing heart (63).

General agreement exists (86, 185, 206, 208) that in failure due to histamine, strophanthin increases the oxygen consumption of the heart, a change which occurs in spite of a reduction in external diastolic volume (86, 185). Since at the same time the work increases to an even greater extent, the mechanical efficiency is increased (86, 185). According to a hypothesis advanced by Rühl (206), the therapeutic action is a consequence of improved supply of oxygen to the myocardium due to facilitation of the diffusion of the gas across the walls of the capillary vessels. The capillary vessel action is held to be responsible also for the improvement of cardiac function in other types of heart failure. Observations on human subjects showing that injection of strophanthin into the cubital artery increases the oxygen uptake in the cubital region are likewise interpreted as indicative of changes in the capillaries favoring the diffusion of oxygen into the tissue (128).

d *Heart Failure Refractory to Cardiac Glycosides* It is not known what influence, if any, the cardiac glycosides may have on cardiac metabolism in heart failure in which they afford no relief, but it is worth recording that they have been found to be of little or no help in disturbances of cardiac metabolism such as myocardial anoxia (127, 129, 154, 223, 245) (but see also 232, 244), thiamine deficiency (71), and thyrotoxicosis (71), in which the contractile power of the myocardium is known or presumed to be weakened by interference with its energy supply. The ineffectiveness of the cardiac glycosides in myocardial anoxia has been interpreted as indicating that they must act during the aerobic phase of contraction (223), but the alternative interpretation, namely, that these drugs restore some non-oxidative process and hence are powerless when the heart fails due to interference with the energy supply for recovery, is just as plausible and is more consistent with what is known about the changes in the energy transformations in the heart during relief of types of failures which respond to cardiac glycosides.

e *Conclusion* The results of the studies examined above do not permit an unequivocal decision as to whether the mechanism of cardiac glycoside action is bound up with energy liberation or with energy utilization. The evidence in favor of improved utilization of chemical energy is strong. At the same time it is clear that energy liberation by the heart may also be increased, apparently independently of other changes, and that this increase is particularly pronounced when the metabolism is depressed in failure. In view of the high specificity of action of the cardiac glycosides and of their effectiveness in trace amounts, it seems unlikely that these drugs relieve different forms of myocardial failure by more than one basic mechanism and that they act directly upon more than one cellular system in the myocardium. That this must be a system of general

abolished or greatly weakened by relatively minor structural alterations. One would expect the relation between structure and cardiac activity to hold for any effect *in vitro* having a bearing on the action *in vivo*.

1 *Fermentation* Incidental observations made during studies on yeast reveal that high concentrations of cardiac glycosides can both stimulate (177) and depress (216) alcoholic fermentation.

Of a different sort are investigations (75, 100, 142, 192, 213, 214, 231) reported on by Freund (72, 73). These investigations, as well as other studies from Freund's laboratory to be discussed below, constitute the first systematic attempt to attack the problem of the mode of action of the cardiac glycosides by the methods of enzyme biochemistry, and herein lies their merit. Data are published according to which "therapeutic" concentrations of strophanthin and digitoxin and their genins ( $1.5 \times 10^6$ – $1.5 \times 10^7$ ) increase the anaerobic glycolysis of minced mammalian heart and skeletal muscle, whereas higher, "toxic" concentrations have as a rule a depressant effect. The anaerobic glycolysis of brain is said to be increased at all concentrations. A particularly pronounced loss of glycolytic power is reported to occur in the heart and skeletal muscle of strophanthin-poisoned animals (231). Freund (73) classifies the cardiac glycosides as agents which modify the carbohydrate metabolism not only of cardiac muscle but of living tissue in general and implies that this constitutes the mechanism of their therapeutic and toxic action on the heart. The fact that the experimental work on which these claims are based was performed by dental and medical students explains perhaps why the results were not confirmed by more responsible investigators.

An inhibition of glycolysis in fresh unwashed horse erythrocytes by strophanthin in concentrations as low as  $1.5 \times 10^5$  has been reported by Segre (218). The inhibition is ascribed to a shift in the lactic acid-pyruvic acid equilibrium in favor of the latter compound, the accumulation of which is stated to cause inactivation of glycolytic enzymes. When the experiment is repeated in the presence of cyanide, strophanthin no longer inhibits but stimulates glycolysis, due to the removal of pyruvate by the keto reagent. These and other effects, to be discussed below, can be obtained in hemolysates, *i.e.*, in the absence of protoplasmic structure.

Profound changes in glycolytic metabolism are produced by the cardiac glycosides in the brain. Weese and Wiegand (242), using slices of guinea pig brain, noted that strophanthin not only strongly inhibits anaerobic glycolysis and moderately depresses respiration, but brings about a qualitative change in the aerobic metabolism which, depending on whether the tabular or the textual presentation of their results is to be believed, constitutes either a pronounced rise of the respiratory quotient ( $R/Q$ ) or a stimulation of glycolysis. Detailed re-investigation of this matter in the same tissue and with additional glycosides (ouabain, digitoxin, scillaren, and others) showed that the  $R/Q$  remains unchanged and that aerobic glycolysis is set in motion (258). At the same time respiration is temporarily increased and anaerobic glycolysis, as in Weese and Wiegand's experiments, permanently depressed. This inhibition of the Pasteur

trations of cardiac glycosides, but skeletal muscle contracture due to various other agents is characterized by an increased oxygen consumption (66)

Recent evidence indicates that depolarization of the muscle fiber membrane is the process which initiates contracture, and propagated contraction as well, and that the depolarized state is required for the maintenance of the contracture (136, 137) From studies on nerve (13, 153) it has become apparent that the energy supplied by oxidative metabolism is the essential factor determining the resting membrane potential and that membrane depolarization will bring about an increase in oxygen consumption In muscle, such a relation could account for at least a fraction of the increased oxygen uptake in contracture, the shortened state of the fibers being possibly another factor favoring increased metabolism (228)

If, for convenience, one considers the membrane changes apart from the energy requirements of the contractile system, the contracture which the cardiac glycosides produce in the heart of cold-blooded animals and in the warm-blooded heart perfused with a salt solution may be assumed to be due to a depolarizing action on the membrane of the myocardial fiber Such a mechanism seems to be responsible for the increased ventricular automaticity (82) The absence of contracture in the warm-blooded heart supplied with blood might then be attributed to the circumstance that under this condition the tissue is able to release energy at a rate sufficient to restore the decreased membrane potential to a level permitting relaxation, hence making possible renewed initiation of contraction The ventricular contracture could thus be regarded as the sequence or the counterpart, respectively, of the increased ventricular automaticity

#### *B Total Energy Yielding Metabolism of Non-Beating Heart Muscle and Other Tissues*

If the cardiac glycosides have an influence on the metabolic activity of the myocardium which is independent of changes produced in mechanical activity—and studies with non-failing frog hearts (p 325) give us reason to believe that this is the case—this influence may be expected to be visible in the non-beating muscle For this reason and in order to study single reaction steps, investigators have turned to experiments with cardiac muscle tissue *in vitro* Other tissues have been used, by some to test for selectivity of action, by others in the hope of demonstrating absence of selectivity since the main pathways of substrate catabolism are largely the same in a great many living cells In evaluating the significance of the results of these studies with regard to the action on the beating heart, two criteria above all have to be applied, namely, the specificity and the order of potency of the agent At high enough concentrations any substance can be expected to affect in some way or other various enzyme proteins and their prosthetic groups The cardiac glycosides, while combining with proteins in the blood plasma (83) and being bound to organs other than the heart (241), have a particularly high affinity for the myocardium, but the effective concentrations in this tissue are extremely low Furthermore, the cardiac activity depends on a number of characteristic structural features of the active molecule and may be



metabolism of minced rat and guinea pig hearts after the addition of strophanthin to give final concentrations ranging from  $1.5 \times 10^7$  to  $1.3 \times 10^8$ . They conclude, and this may be accepted as a fair appraisal of the significance of the preceding work as well, that experiments with minced tissue are not very useful in bringing to light possible metabolic effects of the cardiac glycosides on the beating heart. Attention must be called to the fact that in all the above studies the cardiac tissue preparations were respiring at abnormally low rates.

When, instead of the ill-defined mixture of whole and fragmented cells and free enzymes which goes to make up a fine tissue mince, relatively intact and more steadily respiring pieces of cardiac muscle in the form of slices are used, profound effects of the cardiac glycosides on the respiration become apparent. This has been, with a single exception (101), the common experience in several laboratories as attested by the recent contributions of Lévy and Libert (143, 144, 146, 147), Wollenberger (251, 252, 253, 257, 258), Finkelstein and Bodansky (69) and DuBois *et al.* (57). In general the respiratory action can be obtained at low, pharmacologically relevant concentrations of the cardiac glycosides. Its quality and intensity depend, among other factors, on the concentration of the drug, the composition of the medium, and the species of animal. Increases in the rate of oxygen uptake of as much as 200 per cent (146, 147) as well as total suppression of activity (259) have been observed. Significant changes in the R:Q have not been noticed (253). In buffered Ringer or similar solution containing glucose or lactate, the oxygen consumption is, as a rule, accelerated at all glycoside concentrations, and magnitude and rapidity of this effect are, up to a maximum, proportional to the concentration (69, 251, 253). An analogous proportionality characterizes the decline in the rate of oxygen uptake which at higher than minimal effective concentrations follows the acceleration in cardiac slices of guinea pigs (251, 253) and rats (144). High concentrations of ouabain have been reported to depress the oxygen uptake of rat heart slices in normal medium from the outset (144), but in these experiments readings were taken only once an hour, and it is therefore not unlikely that an initial rise in activity might have been missed. In ventricular muscle slices of the heart of the cat (69) and the dog (251), the sole action of glycosides even at maximal effective concentrations is to produce a rise in respiration lasting for as long as four to five hours. The reason for this species difference is obscure, but in experiments in which only small amounts of dog heart tissues were used (20 mg wet weight or less per 3 cc) the duration of the rise in respiration was reduced and the rate fell below the control level (259). The dependence of all these effects on environmental factors is illustrated by observations that respiration may not be increased and may instead be depressed at once if the incubation medium is altered by acidification (144), omission of substrate (147, 253), or omission of calcium ions (69), and that it has been possible to prevent the inhibition, but not the stimulation, of respiration in guinea pig heart slices by incubating them in boiled heart muscle extracts (252).

So far only one other tissue besides cardiac muscle has been found which responds to cardiac glycosides with an increase in respiration, namely, brain

effect is complete in phosphate-buffered salt solution in which the aerobic glycolysis quickly reaches the normal anaerobic level. None of these changes is seen in cell-free brain preparations or, like the effect of high concentrations of potassium (3, 54) to which they have a striking resemblance, in tissues other than the brain, except that the respiratory action also occurs in heart muscle (cf. below). An inhibition of anaerobic glycolysis has been noticed in heart muscle slices of the rat (147), but not in feebly glycolyzing guinea pig heart slices (258). That stimulation of glycolysis is not noticeable in heart muscle is probably a consequence of the low glycolytic capacity of this tissue (22, 120, 178). The brain effect is also specific in that cardio-inactive derivatives of the cardiac glycosides as well as a number of structurally unrelated cardiac agents are unable to produce it at a concentration ( $2 \times 10^{-5}$  M) which is 5 to 20 times the concentration at which the various cardiac glycosides are highly effective. On the other hand, it can be duplicated with very low concentrations of the structurally related steroid alkaloids protoveratrine and veratridine and the triterpenoid alkaloid coumaringine, all of which have an action on the heart similar to that of the cardiac glycosides (131, 155, 212). Veratrine, the most active component of which is veratridine, has been shown to have a marked stimulating effect on the metabolism of peripheral nerve (215). It is quite possible that the metabolic brain effect is related to the disturbance in the function of the central nervous system seen in digitalis and veratrine poisoning, and the cellular changes in the poisoned brain may be comparable to those in the poisoned heart. Nevertheless, the deduction (242) that the inhibition of anaerobic glycolysis and of respiration in the brain explains the depression of impulse conduction in the heart seems far-fetched.

**2 Respiration** A bewildering mass of data on the influence of cardiac glycosides upon tissue respiration is contained in a series of dissertations from the laboratory of Freund (21, 74, 76, 77, 135, 191, 214). Concentrations of various cardiac glycosides and aglycones of the order of  $1 \cdot 10^3$  to  $1 \cdot 10^7$  are credited with possessing a stimulating and in other cases an inhibiting action on the oxygen uptake of mammalian and frog heart muscle suspended in phosphate buffer. At "toxic" concentrations ( $1 \cdot 10^6$ ) inhibition of the respiratory metabolism is said by some authors (21, 74, 76, 191) to be the predominant effect in these tissues, whereas others (77, 135) insist on observing a stimulant action, in brain and liver an increase in respiration is generally noted at all concentrations. Unless one accepts the existence of radical differences between the individual drugs, there is no way of predicting, from the kind of substrate added or otherwise, the quality or intensity of the action. Of all these findings only the inhibition of respiration in mammalian cardiac muscle, emphasized by Frühauf (76) could be interpreted as being in agreement with the work of later authors (69, 259), though not in the extreme dilution range. But even this action, or any other, is denied by Salomon and Riesser (210) who repeated Frühauf's experiments. Neither do these authors admit any effect of low or of high concentrations of strophanthin when the plain phosphate buffer is replaced by phosphate-Ringer. Genuit and Haarmann (80), too, obtained only insignificant changes in the

metabolism of minced rat and guinea pig hearts after the addition of strophanthin to give final concentrations ranging from  $1.5 \times 10^7$  to  $1.3 \times 10^8$ . They conclude, and this may be accepted as a fair appraisal of the significance of the preceding work as well, that experiments with minced tissue are not very useful in bringing to light possible metabolic effects of the cardiac glycosides on the beating heart. Attention must be called to the fact that in all the above studies the cardiac tissue preparations were respiring at abnormally low rates.

When, instead of the ill-defined mixture of whole and fragmented cells and free enzymes which goes to make up a fine tissue mince, relatively intact and more steadily respiring pieces of cardiac muscle in the form of slices are used, profound effects of the cardiac glycosides on the respiration become apparent. This has been, with a single exception (101), the common experience in several laboratories as attested by the recent contributions of Lévy and Libert (143, 144, 146, 147), Wollenberger (251, 252, 253, 257, 258), Finkelstein and Bodansky (69) and DuBois *et al.* (57). In general the respiratory action can be obtained at low, pharmacologically relevant concentrations of the cardiac glycosides. Its quality and intensity depend, among other factors, on the concentration of the drug, the composition of the medium, and the species of animal. Increases in the rate of oxygen uptake of as much as 200 per cent (146, 147) as well as total suppression of activity (259) have been observed. Significant changes in the R:Q have not been noticed (253). In buffered Ringer or similar solution containing glucose or lactate, the oxygen consumption is, as a rule, accelerated at all glycoside concentrations, and magnitude and rapidity of this effect are, up to a maximum, proportional to the concentration (69, 251, 253). An analogous proportionality characterizes the decline in the rate of oxygen uptake which at higher than minimal effective concentrations follows the acceleration in cardiac slices of guinea pigs (251, 253) and rats (144). High concentrations of ouabain have been reported to depress the oxygen uptake of rat heart slices in normal medium from the outset (144), but in these experiments readings were taken only once an hour, and it is therefore not unlikely that an initial rise in activity might have been missed. In ventricular muscle slices of the heart of the cat (69) and the dog (251), the sole action of glycosides even at maximal effective concentrations is to produce a rise in respiration lasting for as long as four to five hours. The reason for this species difference is obscure, but in experiments in which only small amounts of dog heart tissues were used (20 mg wet weight or less per 3 cc) the duration of the rise in respiration was reduced and the rate fell below the control level (259). The dependence of all these effects on environmental factors is illustrated by observations that respiration may not be increased and may instead be depressed at once if the incubation medium is altered by acidification (144), omission of substrate (147, 253), or omission of calcium ions (69), and that it has been possible to prevent the inhibition, but not the stimulation, of respiration in guinea pig heart slices by incubating them in boiled heart muscle extracts (252).

So far only one other tissue besides cardiac muscle has been found which responds to cardiac glycosides with an increase in respiration, namely, brain

cortex (253) Its sensitivity, however, is considerably lower than that of heart muscle Inhibitory effects on cellular respiration have been observed in a variety of other tissues (57, 253), but these effects are not comparable in intensity to the pronounced inhibition of metabolism which can be produced in slices of brain cortex and cardiac muscle The absence of a specific response in skeletal (146, 253) and smooth (259) muscle is worth noting

Intactness of the structure of the cardiac muscle fiber appears to be a prerequisite for the action of the cardiac glycosides on the respiration of the tissue Ouabain in 100 times the concentration exerting a maximal effect on sliced heart muscle has not the slightest effect on the respiration of the homogenized tissue This has been demonstrated both in concentrated unfortified (253) and in dilute fortified (259) preparations A marked inhibition by ouabain of the respiration of isotonic homogenates of brain has been attributed to the presence of intact cells (253), and this is conceivably also the explanation for a similar inhibitory action of bufagin (57) As a matter of fact, no significant effect of ouabain is seen in hypotonic (253) or in cell-free isotonic (258) brain homogenates It has been concluded (253) that the cardiac glycosides have no *direct* effect on the catalytic activity of oxidative enzymes

The question naturally arises whether the action of the cardiac glycosides on the respiration of heart muscle tissue *in vitro* is of relevance with regard to the action on the beating heart In the light of available evidence, both direct and indirect, this question can without hesitation be answered in the affirmative First of all there is the convincing observation (143, 144, 146, 257) that slices prepared from hearts of animals which have been given injections of cardiac glycosides, in therapeutic to lethal doses, can be distinguished from cardiac muscle slices of control animals by virtue of differences in the rate of oxygen consumption, the first hour values following cardiac glycoside administration being, as a rule, markedly elevated above the control level

Secondly, from what has become known so far about the effect of cardiac glycosides on cardiac tissue respiration, this effect appears comparable in a number of ways to the effect on cardiac function, particularly with respect to its intensity and specificity Thus parallelism is brought out by the following features of the respiratory response (a) The response can be elicited by concentrations and doses of cardiac glycosides which lie within the therapeutic or at least the non-lethal range for the beating heart (69, 251, 253) (b) The order of potency among individual glycosides and aglycones corresponds to that of their positive inotropic and toxic action on the isolated heart (252, 259) (c) The sensitivity of the respiratory response to a given cardiac glycoside varies according to the species of the animal in much the same way as the sensitivity to the inotropic and toxic action of the compound (146) (d) Of all animal tissues tested the myocardium is by far the most sensitive to the respiratory action of the cardiac glycosides and is, besides brain cortex (253), the only one which responds with an increase in respiratory activity (146, 253, 259) (e) The respiratory effect is highly dependent upon certain structural characteristics of the cardiac glycoside molecule which are also required for its typical cardiac action As was found in

brain (258), changes in the molecule which abolish cardiac activity, such as hydrogenation of the lactone ring or allomerization at carbon 17, also abolish the respiratory response (259). Among the metabolically inactive compounds are also simpler unsaturated lactones which produce contracture in the perfused frog heart and several structurally unrelated cardiac agents, among them epinephrine. Again, like in brain (258), the metabolic effect is elicited by low, physiologically relevant concentrations of structurally related alkaloids which have a digitals-like action on the heart, *viz*, the steroid veratrum alkaloids veratridine and protoveratrine and the triterpenoid erythrophleum alkaloid coumaringe (259).

3 *Mechanisms* No satisfactory explanation has been given for the increase or the subsequent decrease in the rate of respiration of cardiac and brain cortex slices by the cardiac glycosides. I suggested at one time (253) that at least a fraction of the increase in respiration is due to increased permeability to exogenous oxidizable substrates and attributed the inhibition of respiration and also of anaerobic glycolysis to a loss of diffusible oxidative catalysts.

Changes in cell permeability have been invoked on a number of occasions, usually on purely speculative grounds, in order to account for unexplained effects of cardiac glycosides. Some evidence of both increases and decreases in membrane permeability to various substances has been obtained in experiments with erythrocytes and model membranes (141, 241). The finding that the fixation of Brilliant Congo Red by the isolated frog heart is slowed in the presence of small amounts of ouabain has been taken as an indication of a decreased permeability of the cardiac muscle fiber (209). On the other hand, abnormal deposition of vital dyes in the myocardium of animals poisoned with digoxin is suggestive of an increased permeability (249), but this change may be entirely incidental to the development of anatomical lesions.

The permeability hypothesis of the stimulant action of the cardiac glycosides on heart and brain tissue respiration is mainly based on experiments (147, 253) showing that in guinea pig and rat heart slices the increase in respiration is proportional, within limits, to the concentration of glucose or lactate in the surrounding medium and does not occur in the absence of these substrates. In support of this hypothesis could also be cited the findings that there is no increase but only a decline of the rate of metabolism when the permeability of the cell is abnormally high to start with, such as in the absence of calcium (69) and in anaerobiosis (147, 252).

That not more than a fraction of the increase in the oxygen uptake caused by cardiac glycosides may be due to accelerated oxidation of exogenous substrate is emphasized by the findings that in cat heart slices this increase is not entirely dependent on the presence of added substrate (69) and that in dog heart slices incubated in glucose-containing solution it is greater than can be accounted for by an increase in glucose uptake, even assuming complete combustion of the extra sugar taken up (259). In the resting ventricular muscle of the frog the stimulating effect of cardiac glycosides on the respiration is entirely independent of the presence or concentration of exogenous glucose (262). These findings, particu-

cortex (253) Its sensitivity, however, is considerably lower than that of heart muscle Inhibitory effects on cellular respiration have been observed in a variety of other tissues (57, 253), but these effects are not comparable in intensity to the pronounced inhibition of metabolism which can be produced in slices of brain cortex and cardiac muscle The absence of a specific response in skeletal (146, 253) and smooth (259) muscle is worth noting

Intactness of the structure of the cardiac muscle fiber appears to be a prerequisite for the action of the cardiac glycosides on the respiration of the tissue Ouabain in 100 times the concentration exerting a maximal effect on sliced heart muscle has not the slightest effect on the respiration of the homogenized tissue This has been demonstrated both in concentrated unfortified (253) and in dilute fortified (259) preparations A marked inhibition by ouabain of the respiration of isotonic homogenates of brain has been attributed to the presence of intact cells (253), and this is conceivably also the explanation for a similar inhibitory action of bufagin (57) As a matter of fact, no significant effect of ouabain is seen in hypotonic (253) or in cell-free isotonic (258) brain homogenates It has been concluded (253) that the cardiac glycosides have no *direct* effect on the catalytic activity of oxidative enzymes

The question naturally arises whether the action of the cardiac glycosides on the respiration of heart muscle tissue *in vitro* is of relevance with regard to the action on the beating heart In the light of available evidence, both direct and indirect, this question can without hesitation be answered in the affirmative First of all there is the convincing observation (143, 144, 146, 257) that slices prepared from hearts of animals which have been given injections of cardiac glycosides, in therapeutic to lethal doses, can be distinguished from cardiac muscle slices of control animals by virtue of differences in the rate of oxygen consumption, the first hour values following cardiac glycoside administration being, as a rule, markedly elevated above the control level

Secondly, from what has become known so far about the effect of cardiac glycosides on cardiac tissue respiration, this effect appears comparable in a number of ways to the effect on cardiac function, particularly with respect to its intensity and specificity Thus parallelism is brought out by the following features of the respiratory response (a) The response can be elicited by concentrations and doses of cardiac glycosides which lie within the therapeutic or at least the non-lethal range for the beating heart (69, 251, 253) (b) The order of potency among individual glycosides and aglycones corresponds to that of their positive inotropic and toxic action on the isolated heart (252, 259) (c) The sensitivity of the respiratory response to a given cardiac glycoside varies according to the species of the animal in much the same way as the sensitivity to the inotropic and toxic action of the compound (146) (d) Of all animal tissues tested the myocardium is by far the most sensitive to the respiratory action of the cardiac glycosides and is, besides brain cortex (253), the only one which responds with an increase in respiratory activity (146, 253, 259) (e) The respiratory effect is highly dependent upon certain structural characteristics of the cardiac glycoside molecule which are also required for its typical cardiac action As was found in

frog ventricular muscle by high concentrations of ouabain precedes the onset of contracture and occurs also in the presence of low concentrations of the glycoside not producing contracture

The increase in the respiration of cardiac muscle tissue even by "non-toxic" amounts of cardiac glycosides has been suspected to be an early sign of tissue damage (253), and at any rate perhaps indicates that some energy requiring process, for example, membrane polarization, is placed under heavy stress. It has not been my belief (253) that this increase in respiration might be responsible for the increase in myocardial contractility in heart failure in the sense that it might furnish extra energy needed for restoring to normal the contractile response. Such a contention would be difficult to reconcile with the finding (see 254) that the cardiac glycosides are fully effective in cases of heart failure in which metabolic energy production is perfectly adequate for keeping the muscle plentifully supplied with ATP and phosphocreatine.

On the other hand, I was tempted to postulate a causal relationship between the inhibition of respiration by the cardiac glycosides in cardiac muscle and their toxic action on the function of the organ (253), having been impressed by the finding that the minimal lethal doses of various cardiac glycosides for the isolated guinea pig heart produce a 50 per cent inhibition of what is probably the basal oxygen consumption of the muscle, and also for the supplementary reason that the anatomical lesions and the electrocardiographic abnormalities seen in the myocardium of animals poisoned with digitals are indistinguishable from those produced by prolonged anoxia (50). This hypothesis is no longer tenable, at least not as far as the acute toxic effects are concerned. The rate of respiration *in vitro* of cardiac muscle of dogs following slow infusion of toxic doses of ouabain is at first much higher than that of cardiac muscle of control dogs (257). Only during the course of prolonged incubation does the rate of respiration fall below the control level. The depression of respiration may be due to additional damage caused by the artificial bathing fluid or other unphysiological conditions of experimentation. The possibility remains that some of the manifestations of chronic cardiac poisoning, including the development of anatomical lesions, may be accompanied or even preceded by a decline in respiratory power. This possibility is, in fact, suggested by the finding of Libert (146) that the oxygen uptake of slices cut from hearts of rats which have been injected with a toxic dose of digitoxin is markedly depressed twenty-four hours following the injection, while it is higher than normal if the animals are sacrificed during the first five hours.

### C Intermediary Metabolism

1 *Carbohydrate Metabolism of the Beating Heart* Gremels (98) reports that the utilization of glucose by the heart in the freshly prepared as well as in the spontaneously deteriorating heart-lung preparation of the dog is markedly increased, at a constant work level, by cardiac glycosides. This effect which can be elicited by as little as 1  $\mu\text{gm}$ , for example, of strophanthoside, is stated to precede the improvement of the efficiency of work and is arbitrarily interpreted as a potenti-

larly the latter, indicate that an increase in permeability to substrates is not the cause, certainly not the most important cause, of the enhanced respiratory activity of cardiac muscle exposed to cardiac glycosides. The increased rate of permeation of glucose into dog and probably into other mammalian heart muscle, in which the cardiac glycoside effect is partly or wholly dependent upon extra cellular substrate, may be merely a consequence and not a cause of the accelerated oxidation of this substrate inside the cell.

The hypothesis that increased permeability, by allowing oxidative catalysts to diffuse out of the cell, is responsible for the inhibitory action of the cardiac glycosides on the metabolism of heart and brain slices fits a number of experimental facts very well, such as the intensification of the inhibitory effect by washing (253), its prevention by contact with boiled heart muscle extract (252), a medium rich in various metabolites and inorganic and organic cofactors, the unimpaired activity of the succinic oxidase system, which functions without pyridine nucleotide, in otherwise severely poisoned cardiac slices (253), and, finally, the inverse relationship between tissue concentration and the rapidity and intensity of respiratory inhibition in heart slices from ouabain-poisoned dogs (257). Interestingly enough, intensification of certain toxic effects of cardiac glycosides by means of washing with Ringer's solution occurs also in contracting skeletal muscle (200), and reversal of the toxic action on the frog heart can be effected by means of washing with extracts of heart muscle (109).

The permeability hypothesis, however, is not helpful in explaining the lack of correlation between tissue concentration and intensity of the inhibitory effect on the metabolism of brain cortex (253), nor does it explain the effectiveness of nicotinamide (0.04 M) and the ineffectiveness of DPN (0.001 M) in preventing the inhibition of anaerobic glycolysis in ouabain-treated brain (258). The latter phenomenon is taken to mean that not outward diffusion, but outright destruction of the coenzyme by its nucleosidase is a cause of the inhibition of glycolysis. Nicotinamide, however, does not protect against inhibition of the aerobic metabolism. Increased DPN-ase activity, therefore, could not be the sole effect of the cardiac glycosides. Other hydrolytic enzymes might conceivably be released from an inactive state. The stimulation of aerobic glycolysis in brain, not explainable on the basis of interference with oxidative phosphorylation (258), likewise points to the removal of a barrier normally restricting cellular enzymatic activity. Changes in the colloidal state of protoplasm, reported to occur in the presence of cardiac glycosides (141, 241), might conceivably be responsible for these effects by making ordinarily inaccessible enzyme surfaces available to their substrates.

Finkelstein and Bodansky (69) have called attention to the possibility that the increased oxygen consumption of cardiac slices incubated in the presence of cardiac glycosides may be associated with contracture of the muscle. Indeed, Victor (233), who reported that ouabain in rather high concentrations greatly increases the oxygen consumption of the resting frog ventricle, found the muscle in a firmly contracted state at the end of his experiments. The contracture hypothesis, however, could not explain the increased oxygen uptake in brain tissue. Furthermore, Yaffe (262) has found that the rise in the oxygen uptake of resting



strophanthin- and digitoxin-poisoned heart-lung preparation. According to Cherkas' interpretation (32) the decrease in lactic acid uptake is due to impairment of its oxidation and its resynthesis to glycogen resulting from myocardial anoxia and does not represent a specific toxic effect of the cardiac glycosides. However, since the oxygen utilization by the digitalis poisoned heart in the heart-lung preparation has been shown to be very high (97, 168), a more likely alternative is that the observed shift in the lactic acid balance is the result of accelerated glycogenolysis, perhaps in response to heavy demands imposed upon the energy providing mechanisms (see also 148).

*2 Isolated Tissues and Oxidative Enzymes* The influence of various cardiac glycosides on the dehydrogenases involved in the oxidation of glucose and some of its intermediaries has been studied by students of Freund (72) by means of the Thunberg technic and using washed cardiac and skeletal muscle minces as sources of the enzymes. The results are highly conflicting. Thus strophanthin in concentrations  $1 \times 10^4$  and higher is reported to have an accelerating action on the anaerobic oxidation of glucose, hexose diphosphate, glycerophosphate and lactate (4), in contrast to inhibitory effects of corresponding concentrations of scillaren and cymarinn (126) and digitoxin and lanadigin (214). Succinate oxidation is stated to be increased by strophanthin (4) and to be unaffected by scillaren and cymarinn (126). Still other qualitative differences between various cardiac glycosides are claimed (149), and finally it is found that adonidine and convallamarin have no significant effect on the various dehydrogenases, even in concentrations up to  $1 \times 10^3$  (183). Only the latter finding is in line with later reports from other laboratories, which indicate that no effect is exerted by ouabain (253) and digitoxigenin (259) on glucose dehydrogenase, and by bufagin (57) on succinic dehydrogenase. On the other hand, it is reported (230) that the anaerobic reduction of m-dinitrobenzene by minced cardiac muscle of rabbits is accelerated by previous digitalization of the animals.

The cytochrome oxidase system appears to be unaffected even by high concentrations of ouabain (253), digitoxigenin (259), and bufagin (57). Neither does the presence of a cardiac glycoside have any influence on the activity of a complete hydrogen and electron transfer system assembled from substrate and its dehydrogenase, oxygen and cytochrome oxidase, and the intervening carriers (253). Inhibition of such a system by a narcotic in a concentration corresponding to that producing severe failure of the isolated heart is not reversed by cardiac glycosides even in 1000 times the concentration in which they relieve this type of heart failure (251). These findings again illustrate, as did the experiments with unpurified enzyme systems in homogenates, that the cardiac glycosides have no direct effect on respiratory enzymes.

Govier and collaborators (92) have reported that minute amounts of digitoxin markedly stimulate the anaerobic lactic dehydrogenase system in homogenates of hearts of vitamin E deficient guinea pigs and have attributed this effect to a protection of DPX, the coenzyme of lactic dehydrogenase, from hydrolysis by its nucleosidase. The same observation was made in anaerobic preparations of rat brain, a tissue high in nucleosidase activity. Curiously enough, exactly the op-

ation of the action of traces of insulin in the blood. A rise of the respiratory quotient of the isolated frog heart accompanying the positive inotropic action of strophanthin in hypodynamy due to ethyl alcohol or lack of calcium points to an increased oxidation of carbohydrate (62).

Mel'nikova (162) finds that the utilization of lactic acid by the heart-lung preparation heart in spontaneous failure is greatly improved by strophanthin concurrently with the restoration of the contractile power of the muscle. The negative lactic acid balance of the heart is reversed and the rate of uptake of the metabolite from the blood eventually exceeds the pre-failure rate. This effect is reflected also in a lowered acidity of the coronary venous blood (85). A sharp rise in the lactic acid uptake by the heart following the administration of non-toxic doses of strophanthin occurs in the heart-lung preparation also in the absence of heart failure (34). It has also been seen in other experiments (14) in which, however, the physiological state of the heart was not determined. The lactic acid utilization of the barbiturate- and avertin-poisoned heart-lung heart, claimed by Rühl (205) to be abnormally high, is according to the same source unchanged following reversal of the poisoning by strophanthin. An increased uptake of lactic acid but not of pyruvic acid has been observed by Mardones and collaborators (159) in the isolated frog heart beating in a medium containing diglucan. Since under the same conditions the oxygen uptake is unchanged, it is concluded that the extra lactate consumed is not oxidized, but is converted to glycogen. This is a debatable conclusion since addition of readily oxidizable substrate can be expected to suppress the oxidation of endogenous metabolites.

Whether or not the cardiac glycosides promote glycogenesis in the heart is not clear from the published data, at least not so far as the therapeutic dose range is concerned. Following the administration of non-toxic doses of cardiac glycosides to rats and rabbits the glycogen content of the heart has been reported to be both increased (2, 32, 33, 263), decreased (18, 148), and not significantly changed (124, 263). Data concerning the effect of non-toxic doses on skeletal muscle are just as conflicting (18, 124, 148). Kimura (124) reports that the glycogen content in the liver of rats given non-toxic doses of digitoxin is elevated, and this effect is due to increased glycogenesis and not to decreased glycogenolysis. Other authors (18, 148) report the liver glycogen to be diminished under similar circumstances. Sharp rises in the glycogen content of the heart and to a lesser extent of the liver have been noted in pigeons treated with a dose of digitoxin bordering on the toxic (105).

In animals manifesting signs of cardiac glycoside poisoning the glycogen stores in the heart (32, 33, 106), liver (124, 264) and skeletal muscle (264) become depleted. Results to the contrary presented by Lasch and Triger (139) are not convincing because excessively high analytical values show that the determinations were not specific for glycogen. Cherkes (32, 33) finds the decrease in the glycogen content of the heart of rats and dogs acutely poisoned with digitoxin and strophanthin to be associated with a correspondingly sharp rise in lactic acid. In conformity with these changes is the diminished uptake or the actual release of lactic acid by the dog heart which he (34) and v. Blumencron (14) find in the

features rather than on characteristics which distinguish one molecule from the other (cf also 43) Of added interest in this connection is that the same basic biochemical change has been implicated, not wholly without experimental support, in both the action of the cardiac glycosides (189) and that of veratrine (88), namely, a displacement of calcium, presumably bound to phospholipids, from the surface of the cell

### *D Energy-Rich Phosphate Content and Transfer*

Non-toxic doses of cardiac glycosides do not produce significant changes in the ATP and phosphocreatine content of the non-failing heart, whether in the intact circulation (124, 259), the Starling heart-lung preparation (255), or the completely isolated Langendorff preparation perfused with Ringer's solution (244) Neither are noticeable changes produced by non-toxic doses in the heart-lung preparation during or following recovery from spontaneous failure or from failure induced by anesthetics (259)

In these types of experimental heart failure, the content of energy-rich phosphates in the heart is normal or even somewhat elevated (p 323), and this fact itself has been taken as a basis for the generalization (254) that the primary action of the cardiac glycosides on the heart must be concerned with the utilization and not with the generation of phosphate bond energy This is also the view adopted by Mardones (159) after evaluation of the pertinent digitalis literature in the light of Szent-Györgyi's (228) theory of muscular contraction and on the basis of his own findings (160, 179) that digitalis glycosides restore the contractility of the isolated rabbit intestine and of the isolated guinea pig heart depressed by phlorhizin, an inhibitor of phosphorylation However, this antagonism to phlorhizin is also open to exactly the opposite interpretation, namely, that phosphorylation may somehow be reactivated by the cardiac glycosides, and hence is best omitted at present as an argument in the discussion

The constancy of the ATP and phosphocreatine levels in the heart treated with a cardiac glycoside merely signifies that the equilibrium between the rates of their breakdown and synthesis is kept at the same level, but it does not tell anything about whether and how the rates of these reactions are changed Direct information on this question is difficult to obtain However, the fact that administration of a cardiac glycoside to the spontaneously failing heart in the heart-lung preparation is known to produce a rise both in oxygen consumption and work performance (185) is strongly indicative of an increased ATP and phosphocreatine metabolism, and this may also be the case in the non-failing heart

As described earlier in this review (pp 315, 323) spontaneous failure of the mammalian heart perfused with Locke solution is associated with a decline in oxygen consumption, phosphocreatine, and possibly ATP, restoration of normal contractility by a cardiac glycoside (strophanthin) is associated with a parallel increase in oxygen consumption Weicker (244) finds the phosphocreatine and ATP content to be likewise restored to normal and the concentration of free adenylic acid to be substantially raised from near zero levels to 50 per cent of the normal value A simultaneous increase in coronary flow points to the possibility that the im-

posite effect of digitoxin and other cardiac glycosides on DPN-ase, namely, its activation, has been postulated to occur in brain slices (p 336) But in contrast to this and other effects of the cardiac glycosides on the metabolism of relatively intact brain and heart tissue, the stimulation of lactate oxidation in the cell free tissue preparations is also obtained with steroids devoid of cardiac activity, such as cholesterol, digitonin and certain sex hormones Nonetheless, Govier *et al* (92) have suggested that the therapeutic action of digitoxin on the failing heart may be due to preservation of DPN This hypothesis no longer has a foundation since the demonstration (224) that DPN-ase activity is not inhibited by digitoxin

Another interesting action on lactic dehydrogenase activity occurs, according to Segre (218), in suspensions of erythrocytes and hemolysates of horse blood In these preparations, strophanthin in a concentration  $1.5 \times 10^5$  or higher is reported to accelerate the oxidation of added lactate and to restore the reaction to normal when inhibited by cyanide The latter effect is explained by attributing to the cardiac glycoside the property of being a hydrogen acceptor, replacing molecular oxygen Strophanthin is believed to function also as a hydrogen carrier, capable, in the same manner as ascorbic acid, of being alternately reduced and oxidized by virtue of a hypothetical reactivity of its unsaturated lactone ring In support of this hypothesis is adduced, in addition to the accelerant effect on lactate oxidation, the finding that aged and repeatedly washed red cells whose glycolytic capacity is diminished, probably largely through destruction or loss of DPN, are enabled by strophanthin to glycolyze glucose at a rate approaching that of fresh, slightly washed cells

Significant as the actual observations may be, these far-reaching inferences are out of proportion to the weight of the evidence presented, and neither are there other data in the literature in support of this line of thought True, ascorbic acid and certain other simple unsaturated lactones have an effect in the isolated frog heart resembling that of the cardiac glycosides, but this effect results from the formation of peroxides (132, 163), the oxidizing action of which possibly interferes with the normal function of sulfhydryl enzymes (164) or perhaps of the sulfhydryl groups of myosin (cf 140) No peroxides have been detected in perfusates of frog hearts treated with cardiac glycosides (130) This still leaves open the possibility that they might be formed at the site of action of the drug in the cell But speculative theories (81) of a mechanism of action of the cardiac glycosides based on the peroxide effect and other properties of unsaturated lactones must face an immediate objection against their implicit assumption of a different mechanism for the cardiac glycoside-like action of certain veratrum and erythrophleum alkaloids, compounds structurally related to the cardiac glycosides, but lacking the lactone ring Yet these compounds share with the cardiac glycosides not only the positive inotropic and the toxic action both on the frog and the mammalian heart (131, 155, 212) but also the characteristic effects on the metabolism of heart and brain tissue *in vitro* (258, 259), and veratridine has been shown to have the same influence as the cardiac glycosides on cardiac energetics (167) It would seem that in attempting to correlate the chemical structure and cardiac activity of these drugs, the emphasis should be placed on common structural

the cardiac glycoside-poisoned heart-lung preparation has been interpreted as reflecting a lag of the oxidative synthesis of ATP relative to its breakdown, which, however, is at once compensated for by phosphate transfer from phosphocreatine. Impairment of the synthesis as well as acceleration of the breakdown of ATP have been proposed as possible causes for the decrease in phosphocreatine (255). Impairment of the synthesis of energy-rich phosphate could result from interference with either oxidation or oxidative phosphorylation. The indications are that in the heart neither of these two processes is interfered with by the cardiac glycosides. The oxygen consumption is, on the contrary, sharply elevated (97, 168, 199) and oxidative phosphorylation in respiring heart muscle homogenates has been found, as in brain (258) and kidney (56), to be unaffected even by high concentrations of cardiac glycosides (259).

There remains the second alternative, namely, that the loss of phosphocreatine in the heart poisoned by a cardiac glycoside is a consequence of an increase in the rate of breakdown of ATP. As was pointed out above, an accelerated breakdown of ATP in all likelihood also accompanies the positive inotropic action of these drugs on the heart. However, as long as there are no extrasystoles and tachycardia, this is not brought to light by chemical analysis because of complete resynthesis of ATP and phosphocreatine during the diastolic pause. Prolongation of diastole, which is usually seen following the administration of therapeutic doses of cardiac glycosides, would thus compensate for the intensification of breakdown processes in systole. Some authors (240) have even gone so far as to declare the lengthened diastole to be the basis of the positive inotropic action of the cardiac glycosides. At any rate, it appears that what at first sight seems to be a purely toxic effect of the cardiac glycosides on the heart, namely, depletion of phosphocreatine, is probably a manifestation of a change associated also with the therapeutic action. Wood and Moe (261) have emphasized that the escape of intracellular potassium, which they (260) and others (25, 104, 220, 239) have noted in hearts poisoned with cardiac glycosides and which most likely is connected to some extent with the metabolism of the organic phosphates in the cell, is likewise a phenomenon characteristic of the therapeutic effect as well. That the potassium content of the heart exposed to therapeutic doses of cardiac glycosides may be undiminished (25, 239) or even increased (19, 104) might be due, like the constancy of the ATP and phosphocreatine levels under the same or similar conditions, to intensification of restorative processes, in this instance to an increase in the rate of reabsorption of the ion.

The possibility that the cardiac glycosides might accelerate the dephosphorylation of ATP through a stimulant action on ATP-ase has been explored by Guerra and collaborators (102). Their data show, notwithstanding the contrary claim of these authors, that ouabain in concentrations of  $1 \cdot 10^{-6}$  to  $1.5 \times 10^{-5}$  has no significant effect on the activity of myosin-ATP-ase preparations from cardiac muscle. It is not inconceivable, however, that the activity of this calcium-activated enzyme might be stimulated by the drug in the intact cardiac muscle, perhaps through mobilization of calcium. According to Kimura and Du Bois (125) digitoxin in a concentration of  $4.7 \times 10^{-6}$  M and ouabain in higher concentrations

provement of the metabolism may be a secondary effect of an improved oxygen supply, which in this heart preparation is at a critical level even under favorable conditions. The observation was made in this study that administration of strophanthin in heart failure due to lack of oxygen sometimes produces a temporary increase in anaerobic work performance which is accompanied by a rise of the phosphocreatine content to the normal level, while the ATP content remains low. This finding suggests that anaerobic synthesis of phosphocreatine may be stimulated by strophanthin. If credence is given to Abdon and Nielsen's (1) claim that strophanthin inhibits the enzymatic hydrolysis of phosphocreatine, Weicker's finding could be interpreted in the sense that the drug prevents an economical dissipation of energy-rich phosphate which, under conditions of failure, might be pronounced. Weicker, however, contents himself with regarding the anaerobic as well as the aerobic action of strophanthin on the phosphocreatine metabolism of the heart as secondary to unknown physicochemical changes favoring anabolic activity.

Ide's (116) report that ouabain restores to normal the depleted phosphocreatine content of the hypodynamic heart cannot be evaluated since no information is given as to the origin of failure and the experimental procedures.

The finding of Herrman and Decherd and collaborators (52, 114) that digitalization of rabbits with experimental cardiac hypertrophy leads to significant increases in the creatine content of the injured hearts is of interest, but whether this indicates, as the authors imply (113), a corresponding increase in phosphocreatine must be questioned for reasons given on p. 324.

The ATP and phosphocreatine content of the frog's heart is not significantly changed by poisoning the animal with cardiac glycosides (31), but the toxic action of the drugs on the mammalian heart is characterized, particularly in its advanced stages, by a depletion of the energy-rich phosphate store. In the isolated cat heart perfused with Ringer's solution, contracture-producing doses of strophanthin cause a 50 per cent loss of phosphocreatine and an even greater reduction of ATP (244). Such disappearance of energy-rich phosphate in muscle is a characteristic feature of all kinds of contractures and rigor (166, 228). In the heart-lung preparation of the dog poisoned with ouabain or digoxin, a progressive decrease of phosphocreatine sets in with the appearance of extrasystoles and ventricular tachycardia, and at the onset of ventricular fibrillation 75 per cent of the amount originally present has disappeared (255). A similar depletion is seen in the intact animal (259). The loss of phosphocreatine is not a consequence of tachycardia *per se*, since the same increase in heart rate produced by electrical stimulation or by epinephrine is ineffective, it represents, therefore, a specific effect of the cardiac glycosides. The ATP content of the poisoned dog heart, whether *in situ* or in the heart-lung preparation, is not significantly altered by poisoning with cardiac glycosides. It is not possible to say whether this has any bearing on the fact that, in contrast to the ATP-depleted Langendorff preparation, the heart under these conditions rarely goes into contracture.

In conformity with the view that phosphocreatine functions as an energy reservoir for the adenylic system (150), the observed loss of phosphocreatine in

the cardiac glycoside-poisoned heart-lung preparation has been interpreted as reflecting a lag of the oxidative synthesis of ATP relative to its breakdown, which, however, is at once compensated for by phosphate transfer from phosphocreatine. Impairment of the synthesis as well as acceleration of the breakdown of ATP have been proposed as possible causes for the decrease in phosphocreatine (255). Impairment of the synthesis of energy-rich phosphate could result from interference with either oxidation or oxidative phosphorylation. The indications are that in the heart neither of these two processes is interfered with by the cardiac glycosides. The oxygen consumption is, on the contrary, sharply elevated (97, 168, 199) and oxidative phosphorylation in respiring heart muscle homogenates has been found, as in brain (258) and kidney (56), to be unaffected even by high concentrations of cardiac glycosides (259).

There remains the second alternative, namely, that the loss of phosphocreatine in the heart poisoned by a cardiac glycoside is a consequence of an increase in the rate of breakdown of ATP. As was pointed out above, an accelerated breakdown of ATP in all likelihood also accompanies the positive inotropic action of these drugs on the heart. However, as long as there are no extrasystoles and tachycardia, this is not brought to light by chemical analysis because of complete resynthesis of ATP and phosphocreatine during the diastolic pause. Prolongation of diastole, which is usually seen following the administration of therapeutic doses of cardiac glycosides, would thus compensate for the intensification of breakdown processes in systole. Some authors (240) have even gone so far as to declare the lengthened diastole to be the basis of the positive inotropic action of the cardiac glycosides. At any rate, it appears that what at first sight seems to be a purely toxic effect of the cardiac glycosides on the heart, namely, depletion of phosphocreatine, is probably a manifestation of a change associated also with the therapeutic action. Wood and Moe (261) have emphasized that the escape of intracellular potassium, which they (260) and others (25, 104, 220, 239) have noted in hearts poisoned with cardiac glycosides and which most likely is connected to some extent with the metabolism of the organic phosphates in the cell, is likewise a phenomenon characteristic of the therapeutic effect as well. That the potassium content of the heart exposed to therapeutic doses of cardiac glycosides may be undiminished (25, 239) or even increased (19, 104) might be due, like the constancy of the ATP and phosphocreatine levels under the same or similar conditions, to intensification of restorative processes, in this instance to an increase in the rate of reabsorption of the ion.

The possibility that the cardiac glycosides might accelerate the dephosphorylation of ATP through a stimulant action on ATP-ase has been explored by Guerra and collaborators (102). Their data show, notwithstanding the contrary claim of these authors, that ouabain in concentrations of  $1 \cdot 10^6$  to  $1 \cdot 5 \times 10^5$  has no significant effect on the activity of myosin-ATP-ase preparations from cardiac muscle. It is not inconceivable, however, that the activity of this calcium-activated enzyme might be stimulated by the drug in the intact cardiac muscle, perhaps through mobilization of calcium. According to Kimura and Du Bois (125) digitoxin in a concentration  $4 \cdot 7 \times 10^{-6}$  M and ouabain in higher concentrations

are capable of inhibiting the splitting of ATP in cardiac muscle preparations by a phosphatase not activated by calcium, possibly Kielley and Meyerhof's (122) magnesium-activated ATP-ase

An indication that the cardiac glycosides may have an influence, at least under certain conditions, on the interaction of actomyosin and ATP is given in the announcement by Mallov and Robb (156) of their finding that actomyosin preparations treated with a cardiac glycoside in a concentration of  $1:2 \times 10^{-6}$  yield threads which, in contrast to threads drawn from untreated preparations, exhibit, in the presence of ATP and magnesium, considerable "relaxation" followed by maximal shortening. It would be of interest to know whether this effect of the cardiac glycoside is restricted to substances which enhance the contractile power of cardiac and other muscle. In any case, whatever the significance of the observations of Mallov and Robb may prove to be, the statement made by one of the authors (201) in reviewing recent literature on cardiac biochemistry and metabolism<sup>3</sup>, that "after some two hundred years, almost any day, the complete mechanism of cardiac glycoside action may become known," seems unduly optimistic. Much progress remains to be achieved in elucidating the basic molecular events in muscular activity, before there will be justification for stating that the problem of elucidating the mechanism of action of agents such as the cardiac glycosides is about to be solved.<sup>4</sup>

#### REFERENCES

- 1 ADDON, N O AND NIELSEN, N A The influence of atropine and acetylcholine on the enzymatic breakdown of phosphocreatine. *Scandinav Arch f Physiol*, 78: 13-16, 1936
- 2 D'ALESSANDRIA, E Influenza del cardiotonico sul glicogeno del muscolo cardiaco. *Folia med.*, 18: 1399-1406, 1932.
- 3 ASHFORD C A AND DIXON, K C The effect of potassium on the glycolysis of brain tissue with reference to the Pasteur effect. *Biochem J*, 29: 157-168, 1935
- 4 AVERBUCK, B Über den Einfluss von Strophanthin auf die Methylenblaufärbung. Inaugural dissertation, 15 pp., Universität Münster, 1932
- 5 BAKER, J B E The influence of calcium and potassium ions on the toxicity of ouabain. *Brit. J Pharmacol. Chemother.*, 2: 259-267, 1947
- 6 BALL, E G AND COOPER, O The activity of succinate oxidase in relation to phosphate and phosphorus compounds. *J Biol Chem*, 180: 113-124, 1949
- 7 BATLISA, L E, MÖLLER, E A AND STARLING, E H The action of insulin and sugar on the respiratory quotient and metabolism of the heart lung preparation. *J Physiol*, 65: 33-47, 1928
- 8 BEATTIE, F, MILROD, T H AND STRAIN, R. W M The nucleotide component of mammalian cardiac muscle. I. The chemical composition and chemical properties of the nucleotide. *Biochem. J*, 28: 84-90, 1934.
- 9 BERG, H Über den Herzmuskelstoffwechsel bei Hyperthyreose und seine Beeinflussung durch Vitamin C. *Arch. f exper Path u Pharmacol*, 185: 359-367, 1937
- 10 BERLINER, K The effect of calcium on the heart. *Am. Heart J*, 8: 348-362, 1933-33

<sup>3</sup> In this otherwise very informative review several of the findings of the present author are unfortunately not correctly represented

<sup>4</sup> As this review goes to press, the author has learned of renewed attempts to relate the positive inotropic action of the cardiac glycosides on the failing heart to an increase in ATP-ase activity said to have been observed in muscle homogenates (R Hegglin, H Grauer, and R Münchinger, *Experientia*, 5: 127, 1949; G Segre, *Arch int pharmacodyn*, 80: 336-436, 1949). The suppression of aerobic glycolysis in the stomach musculature of the guinea pig by low concentrations of ouabain is claimed (G Werner, *Arch int pharmacodyn*, 79: 323-331, 1949). K P DuBois (personal communication) finds that bufagin produces the same kind of stimulation of the respiration of guinea pig heart slices as do digitoxin and strophanthin. E C del Pozo and E G Pardo (*J Pharmacol and Exper Therap*, 97: 144-149, 1949) report that the amplitude of contraction of ischemic skeletal muscle is increased following intravenous injection of k strophanthoside



11. BERNHEIM, F AND BERNHEIM, M. L. C. The effect of various conditions on the respiration of rat heart muscle in vitro. *Am. J. Physiol.*, 142: 195-199 1944.
12. BING R J., HAMMOND M M., HANDLISMAN J C. POWERS, S. R., SPENCER, F C. ECKENHOFF J E., GOODALE, W T. HAFKENSCHIL, J H AND KETY S S. The measurement of coronary blood flow oxygen consumption and efficiency of the left ventricle in man. *Am. Heart J.*, 38 1-24, 1949
13. BLAIR H. A. On the kinetics of recovery during the refractory period in frog's nerve. *J. Neurophysiol.*, 1 127-143 1938
14. BLUMENKROV W. FRH. v. Über die Wirkung von Strophanthin und Digitoxin auf den Milchsäurestoffwechsel des Herzens. *Klin. Wchnschr.* 20 737-739, 1941
15. BONANSKY M AND PILCHER, J F. Clinical significance of the creatine reserve of the human heart. *Arch. Int. Med.*, 59 232-244 1937
16. BOOVE, J Y., CHANO I. AND GREGORY R. A. Metabolism of isolated mammalian heart under partial anoxia. *Quart. J. Exper. Physiol.* 27 319-341, 1938
17. BOOVE J Y. AND MENDEZ, R. The relation between the mechanical and electrical response of the frog's heart. *J. Physiol.* 69 316-330 1930
18. BOMSKOV C AND KAULLA K. N. v. Über die Wirkung von Sternnderivativen auf den Stoffwechsel. II. Über die Wirkung der Digitalisubstanzen auf Leber, Herz und Muskelglykogen. *Arch. f. exper. Path. u. Pharmacol.* 198 233-244 1941
19. BOYER, P. K. AND POINTDEXTER, C. A. The influence of digitalis on the electrolyte and water balance of heart muscle. *Am. Heart J.*, 20 586-591 1940
20. BRAUN-MENDEZ, E. CHUTE A L AND GREGORY, R. A. Usage of pyruvic acid by dog's heart. *Quart. J. Exper. Physiol.*, 29 91-104 1939
21. BUDDEBERG H. Über den Einfluss der Digitalisstoffe Digitalin, Digitaligenin und Gitoxin auf biologische Oxydationsvorgänge. Inaugural dissertation, 27 pp. Universität Münster 1933
22. BUZZI, M V., STRACAS M B AND ANDREAS E C. Metabolic changes involving phosphorus and carbohydrate in the autolyzing gastrocnemius and cardiac muscles of normal, of thyroxinized, and of adrenalectomized animals. *J. Biol. Chem.* 98 645-669 1933
23. BURKE, W. AND CRUKSHANK, E. W. H. Changes in creatine phosphagen and adenylypyrophosphate in relation to the gaseous metabolism of the heart. *J. Physiol.*, 91 314-323 1937
24. CALDER, R. M. Effect of nicotinic acid on myocardial systole, coronary flow, and arrhythmias of isolated heart. *Proc. Soc. Exper. Biol. and Med.* 65 76-83 1947
25. CALHOUN J. A. AND HARRISON, T. R. The effect of digitalis on the potassium content of cardiac muscle of dog. *J. Clin. Investigation* 9 393-403 1931
26. CATTLE, McK. The influence of ouabain on the contraction of striated muscle. *J. Pharmacol. and Exper. Therap.*, 62 459-469 1933
27. CHANO, I. Asphyxial arrest of the isolated rabbit's auricle. *Quart. J. Exper. Physiol.*, 27 113-131 1937
28. CHANO, I. Effect of asphyxia on the adenosinetriphosphate content of the rabbit's heart. *Quart. J. Exper. Physiol.*, 28 3-13 1933
29. CHANO, I. AND LI L. The serohlo breakdown and reynthesis of phosphagen in the rabbit's heart. *Chinese J. Physiol.* 16 265-276, 1941
30. CHEN, G. AND GEILING, E. M. K. The effect of thiamine-deficiency, quinidine hyperthyroidism and hypothyroidism on the adenosinetriphosphate content and the adenosinetriphosphatase activity of the heart muscle of rats. *Fed. Proc.*, 5 169-170 1946.
31. CHEN, G. AND GEILING E. M. K. Some aspects of the biochemistry and pharmacology of the heart. *Schweiz. med. Wchnschr.*, 77 25-28, 1947
32. CHERKES A. I. Herzmittelwirkung und Herzstoffwechsel. *Acta med. URSS*, 3 155-167 1940.
33. CHERKES A. I. AND ANOARSKAYA, M. A. Eksperimentalnye issledovaniya po farmakologii serdca. I. Deystvie strophantina na uglevodnyy obmen serdechnoi myshitsy (Experimental studies in the pharmacology of the heart. I. Effect of strophanthin on the carbohydrate metabolism of the cardiac muscle.) *Farmakol. i Toksikol.*, 2: 60-70 1939
34. CHERKES A. I. AND MEL'NIKOVA V. F. Vliyaniye strophantina na obmen molodnoy kisloty v serdce (Effect of strophanthin on the exchange of lactic acid in the heart.) *Bull. Eksp. Biol. Med.*, 13 42-46 1942.
35. CLARK, A. J. The action of ions and lipids upon the frog's heart. *J. Physiol.*, 47 66-107 1913-14.
36. CLARK A. J. AND EGGLETON M. G. Phosphagen changes in the iodoacetate poisoned frog's ventricle. *Quart. J. Exper. Physiol.*, 26 119-126, 1937
37. CLARK, A. J., EGGLETON M. G. AND EGGLETON P. Phosphagen in the perfused heart of the frog. *J. Physiol.* 75 332-344 1932
38. CLARK, A. J. EGGLETON M. G. EGGLETON P., GARRIE, R. AND STEWART, C. P. The metabolism of the frog's heart. XII and 303 pp. Oliver and Boyd Edinburgh and London 1933
39. CLARK, A. J. AND WHITE, A. C. Oxygen consumption of the frog's heart. II. Effect of lipids, ions and narcotics. *J. Physiol.*, 66 202-216 1923
40. CLARK, A. J. AND WHITE, A. C. Action of ionic changes on the oxygen consumption of the frog's auricle. *J. Physiol.* 68 433-440, 1923-30.
41. CONSTABLE F. Über den Kreatingehalt des menschlichen Herzmuskels bei verschiedenen Krankheitszuständen. *Biochem. Ztschr.* 122 152-153 1921
42. COWAN, D. W. The creatine content of the myocardium of normal and abnormal human hearts. *Am. Heart J.*, 9 378-385, 1933-34.

- 43 CRAIG L. C. AND JACOBS W. A. The veratrine alkaloids. XIV The correlation of the veratrine alkaloids with the solanum alkaloids. *Science*, 97 122, 1943
- 44 CRUKSHANK, E. W. H. Cardiac metabolism. *Physiol. Rev.*, 16 597-639, 1936
- 45 DALY, I. DEB. AND THORPE, W. V. The isolated mammalian heart preparation capable of performing work for prolonged periods. *J. Physiol.*, 79 199-217, 1933
- 46 DANIELOPOLU, D. La digitale et les strophanthines considérées comme médicaments favorisant les médiateurs chimiques. *Cardiologia*, 12 66-53, 1947
- 47 DAVID, J. C. The action of drugs on the oxygen consumption of the frog's isolated auricle. *J. Pharmacol. and Exper. Therap.*, 40 229-234, 1930
- 48 DAVIES, F., FRANCIS, E. T. B. AND STONES, H. B. The distribution of nucleotide, phosphocreatine and glycogen in the heart. *J. Physiol.*, 106 154-166, 1947
- 49 DE, P. The rate of action of drugs and ions on frog's heart. *J. Pharmacol. and Exper. Therap.*, 33 115-123, 1925
- 50 DEARNO, W. H., BARNES, A. R. AND ESSEX, H. E. Experiments with calculated therapeutic and toxic doses of digitalis. VI Comparative effects of toxic doses of digitalis and prolonged deprivation oxygen on the electrocardiogram, heart, and brain. *Am. Heart J.*, 27 108-120, 1944
- 51 DECHERD, G. M. AND BLUM, J. E. Phosphorus fractions in human heart muscle. *Proc. Soc. Exper. Biol. and Med.* 38 341-343, 1938
- 52 DECHERD, G. M., HERMANN, G. AND SCHWAB, E. H. Experimental hypertrophy without and with digitalization on creatine content of rabbit hearts. *Proc. Soc. Exper. Biol. and Med.*, 34 864-866, 1936
- 53 DECHERD, G. M. AND VISCHEK, M. B. Energy metabolism of the failing heart. *J. Exper. Med.*, 59 195-199, 1934
- 54 DICKENS, F. AND GREVILLE, G. D. The metabolism of normal and tumor tissue. XIII Neutral salt effects. *Biochem. J.*, 29: 1468-1483, 1935.
- 55 DOCK, W. Capacity of coronary bed in cardiac hypertrophy. *J. Exper. Med.*, 74: 177-186, 1941
- 56 DUBOIS, K. P. Personal communication
- 57 DUBOIS, K. P., DOULL, J. AND GERTING, E. M. K. Inhibitory action of hufagin on carbohydrate oxidation. *Fed. Proc.*, 8 287-288, 1949
- 58 DUBUASON, M. Les conceptions actuelles de la contraction musculaire. *Experientia*, 3. 213-223, 1947
- 59 DUBUASON, M. AND JACOB, J. L'électrophorèse des protéines des muscles striés, normaux et fatigués, de la grenouille. *Rev. Canad. Biol.*, 4 426-451, 1945
- 60 DEUTSCHE, H. J. Kolloidzustandsänderungen der Muskelproteine beim Absterben und bei der Ermüdung. *Arch. f. d. ges. Physiol.*, 224: 1-43, 1930.
- 61 EISMAYER, G. AND QUINCKE, H. Stoffwechseluntersuchungen am Kaltblütlerherzen. III. Mitteilung: Sauerstoffverbrauch, Kohlensäure- und Milchsäure-Abgabe des Herzens bei verschiedener Arbeit. *Ztschr. f. Biol.*, 89: 523-535, 1930.
- 62 EISMAYER, G. AND QUINCKE, H. Stoffwechseluntersuchungen am Kaltblütlerherzen. V. Mitteilung: Einfluss von Strophanthin und Hexeton auf Atmung und Arbeit des Herzens. *Arch. f. exper. Path. u. Pharmacol.*, 150: 308-315, 1930.
- 63 EVANS, C. L. The metabolism of cardiac muscle. In Evans, C. L., *Recent Advances in Physiology*, 6th Ed., 157-215. Blakiston's Son and Co., Philadelphia 1939
- 64 EVANS, C. L., GRANDT, F. AND HAU, F. Y. The glucose and lactate consumption of the dog's heart. *Quart. J. Exper. Physiol.*, 24 347-364, 1935
- 65 FAHR, G. AND BUEHLER, M. S. A physiologic definition of acute congestive heart muscle failure. *Am. Heart J.*, 25 211-241, 1943
- 66 FENN, W. O. The oxygen consumption of frog muscle in chemical contractures. *J. Pharmacol. and Exper. Therap.*, 42 81-97, 1931
- 67 FENN, W. O. Contractility. In Höber, R., *Physical Chemistry of Cells and Tissues*, 445-522. The Blakiston Company, Philadelphia and Toronto, 1945
- 68 FIESCHI, A. AND GAVAZZENI, M. Sul chimismo cardiaco. Nota VI. Fosfogeno, fosforo e creatina nel miocardio corso della tireotossicosi acuta. *Riv. di pat. esper.*, 9 17-22, 1932
- 69 FINKELSTEIN, M. AND BONAWSKY, O. The effect of cardiac glycosides on the respiration of cardiac muscle. *J. Pharmacol. and Exper. Therap.*, 94 274-287, 1948
- 70 FISCHER, W. Untersuchungen über die Wirkung kleinster Gaben von Äthylalkohol auf das isolierte Herz. *Arch. f. exper. Path. u. Pharmacol.*, 80 93-130, 1917
- 71 FISHBERG, A. M. *Heart Failure*. 2nd Ed., 829 pp. Lea and Febiger, Philadelphia, 1940
- 72 FREUND, H. Digitaliswirkung und stoffwechsel. *Arch. f. exper. Path. u. Pharmacol.*, 167 73-76, 1932
- 73 FREUND, H. Über die Muskelwirkung der Digitalisstoffe. *Arch. f. exper. Path. u. Pharmacol.*, 180 224-230, 1936
- 74 FRIEDEL, W. Über den Einfluss von Digitalin, Digitoxinin und Gitalin auf die Sauerstoffzehrung der Gewebe. Inaugural dissertation, 23 pp., Universität Münster, 1933
- 75 FRIEDRICH, W. Über das Verhalten des Glukose- und Glykogenabbaus unter der Einwirkung des Digitalisglykosides Digitalin und seines Gennas Digitaligenin. Inaugural dissertation, 18 pp., Universität Münster, 1933
- 76 FRIEDHAUF, T. Über den Einfluss von Strophanthin auf die Sauerstoffzehrung. Inaugural dissertation, 15 pp., Universität Münster, 1932
- 77 FRYE, M. Über die Kombinationswirkung von Coffein und Strophanthin auf die Sauerstoffzehrung überlebender Gewebe. Inaugural dissertation 20 pp., Universität Münster, 1935
- 78 GABER, H. S. Contractures of skeletal muscle. *Physiol. Rev.*, 10 35-109, 1930
- 79 GAUER, O. AND KRAUER, K. Zur Frage der oxydationssteigernden Wirkung des Adrenalins am Warmblütlerherzen. *Arch. f. d. ges. Physiol.*, 242 328-343, 1939

50. GENUY H AND HAARMANN W Zur Frage der Spezifität der Digitalisstoffwechselwirkungen an Herz und Skelettmuskulatur von Ratte und Meeresschweinchen. Arch f exper Path. u Pharmacol. 196 431-432 1940
51. GLASMAN, N J Antibiotic lactones and synthetic analogs II Cardiotonic effects on the isolated frog heart J Pharmacol and Exper Therap 96 119-134, 1949
52. GOLDENBERG, M. AND ROTHSBERGER, C Untersuchungen an der spezifischen Muskulatur des Hundeherzens. Arch. f d. ges. Physiol. 236 277-297 1935
53. GOLDBSTEIN A. The interactions of drugs and plasma proteins Pharmacol Rev 1 103-165 1949
54. GOLWITZER MEIER, K. Die Energetik des Säugetierherzens. Klin. Wchnschr., 18 225-231, 1939
55. GOLWITZER MEIER, K. Über die Reaktionsveränderungen im Herzen während der Belastung bei Herzinsuffizienz und unter dem Einfluss von Strophantin Arch f d. ges. Physiol. 245 385-397 1941
56. GOLWITZER MEIER, K. AND KROGER, E. Herzerenergetik und Strophantinwirkung bei verschiedenen Formen der experimentellen Herzinsuffizienz. Arch. f d. ges. Physiol. 238 251-278, 1936
57. GOLWITZER-MEIER, K. AND KROGER, E. Einfluss der Herznerven auf den Gasstoffwechsel des Herzens. Arch. f d. ges. Physiol., 240- 59-110 1939
58. GORDON, H. T. AND WELCH, J. H. The role of ions in axon surface reactions to toxic compounds. J. Cell. and Comp. Physiol., 31 395-420 1948
59. GORTDENKER, F. AND ROTHSBERGER, C. J. Über den Milchsäurestoffwechsel des Herzens bei spontaner Insuffizienz und bei Vergiftung mit Natriumfluorid, Monojodessigsäure und Euphyllin. Arch f d. ges. Physiol. 237 59-67, 1937
60. GORTSCHALK, G. Über die Wirkung des Strophantins auf den Sauerstoffverbrauch des Froschherzens. Arch. f exper Path. u. Pharmacol. 75 33-42, 1913
61. GOVIER, W. M. The effect of experimental coronary arterial ligation on the coenzyme I and co-carboxylase content of the myocardium of the dog. Am Heart J, 29 334-359 1945
62. GOVIER, W. M., YANG N. AND GELLS, M. E. The effect of  $\alpha$  tocopherol phosphate, digitoxin and certain compounds related to the latter on cardiac muscle metabolism in vitro J Pharmacol. and Exper Therap., 88 373-381, 1946.
63. GRAUER, H. Über die Bedeutung des Glykogens im Herzmuskel. Cardiologia, 12. 29-45, 1947
64. GRAUER, H. AND HEGGLIN R. Experimentelle Untersuchungen über die sog. energetisch-dynamische Herzinsuffizienz. Cardiologia, 11 1-35, 1946.
65. GRENZELS, H. Zur Physiologie und Pharmakologie der Energetik des Säugetierherzens. Arch. f exper Path. u. Pharmacol. 169 639-723 1933
66. GRENZELS, H. Über die Steuerung der energetischen Vorgänge am Säugetierherzen. Arch. f exper Path. u. Pharmacol., 182. 1-54 1936
67. GRENZELS, H. Über den Einfluss von Digitalisglykosiden auf die energetischen Vorgänge am Säugetierherzen Arch. f. exper Path. u. Pharmacol., 186 625-660 1937
68. GRENZELS, H. Über die Beziehungen des Kohlehydratstoffwechsels zur Physiologie und Pharmakologie der Herzerenergetik. Arch. f exper Path. u. Pharmacol., 194: 629-690 1940.
69. GRENZELS, H. AND STARLING, E. H. The influence of hydrogen ion concentration and of anoxemia upon the heart volume. J Physiol. 61 297-304 1926
70. GROSS-SILBECK, W. Über den Einfluss des Lanatglykosides Lanadign und seines Aglykons Lanadigenin auf den anseroben Kohlenhydratabbau. Inaugural dissertation 15 pp., Universität Münster 1933
71. GUERRA, F. La significación del consumo ventricular de oxígeno y la liberación enzimática de fosforo en la mecánica de acción de los digitálicos. Memorias del Segundo Congr. Internam. de Cardiol. 399-401, 1946
72. GUERRA, F., EBERSTADT P. L. AND VERRAMP, A. Acción de la uabafina sobre la liberación enzimática de fosforo en el sistema miosina fosfato de adenosina. Arch. Inst. Cardiol. Mexico 16 449-453 1946.
73. GUTTMANN, S. A. AND CATTELL MCK. The effects of digitoxin and potassium on striated muscle J Pharmacol. and Exper Therap 68 267-277, 1940.
74. HAGEN P. The effects of digitanid C in varying dosage upon the potassium and water content of rabbit heart muscle. J Pharmacol and Exper Therap., 67 50-55, 1939
75. HAHN, F. Änderungen im Glykogengehalt des Herzens als Zeichen kumulativer Digitaliswirkung. Arch. f exper Path. u. Pharmacol., 194. 62-73, 1939
76. HÄNDER, M. AND MÜLLER, A. Studien über den Stoffwechsel des Herzmuskels I Mitteilung Über das Herzglykogen. Biochem. Ztschr. 212: 35-46, 1929
77. HARRISON T. R. Failure of the Circulation, 2nd Ed., XIV and 502 pp. Williams and Wilkins, Baltimore, 1939
78. HARRISON, T. R., FRIEDMAN B. AND REINER, H. Mechanism of acute experimental heart failure Arch. Int. Med. 57 427-443 1936
79. HÄUSER, H. Über Digitalisentgiftung. Arch. f exper Path. u. Pharmacol. 190 212-213 1938
80. HEGGLIN R. Die energetisch-dynamische Herzinsuffizienz eine besondere Form der Myokardschwäche. Schweiz. Med. Wchnschr. 25 1104-1109, 1944.
81. HEGGLIN R. Die Klinik der energetisch-dynamischen Herzinsuffizienz Bibliotheca Cardiol. Fasc. 2 120 pp., 1947
82. HEGGLIN R. AND GRAUER, H. Über Beziehungen des Glykogengehaltes im Myokard zur energetischen Herzinsuffizienz. Helvet. med. acta 13 356-362, 1946.
83. HERRMANN, G. AND DECHARD G. M. The chemical nature of heart failure. Ann. Int. Med. 12 1233-1244 1939
84. HERRMANN, G. DECHARD G., SCHWAR, E. H. AND EHRHARD P. Creatine content of digitalized normal and hypertrophied rabbit heart muscle. Proc. Soc. Exper. Biol. Med. 33 522-524 1936
85. HENWICH H. E., GOLDFARB W. AND NARUM H. L. Changes of the carbohydrate metabolism of the heart following coronary occlusion. Am J Physiol. 109 403-403 1934

- 116 IDZ, S Über den Stoffwechsel bei Funktionsstörungen des Herzens. Hukuoka Acta Med , 33 35-38, 1940.
- 117 JACOB, J Etude électrophorétique des variations de composition d'extraits musculaires de Lapin sous l'influence de la fatigue et de la contraction par le monobromacetate de soude. Experientia, 3: 241-243, 1947
- 118 KATZ, L. N Observations on cardiac failure and the mode of its production In Publication 13 of Am. Assn. Adv Sci Blood, heart and circulation 184-194, Washington, 1940.
- 119 KATZ, L. N Personal communication.
- 120 KATZ, L. N AND LONG, C. N. H Lactic acid in mammalian cardiac muscle. I The stimulation maximum. Proc. Roy Soc., London, s B, 99 8-20, 1926.
- 121 KATZ, L. N AND MENDLOWITZ, M Heart failure analyzed in the isolated heart circuit. Am. J. Physiol., 121: 282-273 1938
- 122 KIELLEY, W. W AND MEYERHOF, O Studies on adenosinetriphosphatase of muscle. II A new magnesium-activated adenosinetriphosphatase. J. Biol. Chem., 176 591-601, 1948
- 123 KIESE, M AND GABAN, R. S Mechanische Leistung, Grösse und Sauerstoffverbrauch des Warmblüterherzens. Arch f exper Path u Pharmacol, 183 226-245, 1938
- 124 KIMURA, T. E Effect of cardiac drugs on the phosphorylated intermediates of the rat. Proc. Soc. Exper Biol. and Med., 69 419-422, 1948
- 125 KIMURA, T. E AND DOBBS, K. P Inhibition of the enzymatic hydrolysis of ATP by certain cardiac drugs. Science, 106 370-371, 1947
- 126 KIRCHNER, F Über den Einfluss der digitalisartigen Glykoside Scillaren und Cymarin auf die Dehydrationsvorgänge im Gewebe. Inaugural dissertation, 14 pp., Universität Münster, 1933
- 127 KISCH, F AND SCHWARZ, H Zur Wirkung des Strophanthins bei Sauerstoffmangel. Arch f exper Path. u. Pharmacol, 181 557-562, 1936
- 128 KLEIN, O AND GORSCH, K. Über die Wirkung des Strophanthins auf den inneren Gaswechsel bei intraarterieller Injektion. Ztschr f d. ges. exper. Med., 101 219-233, 1937
- 129 KÖNIG, W Herzarbeit ohne Sauerstoff III Mitteilung. Herzmittelwirkung am Warmblüterherzen. Arch. f. exper Path u Pharmacol., 127 349-365, 1927
- 130 KRAYER, O Unpublished experiments.
- 131 KRAYER, O AND ACHESON, G. H. The pharmacology of the veratrum alkaloids. Physiol Rev 26 383-448, 1946.
- 132 KRAYER, O, LUNSTAD, R. P. AND TONN, D Pharmacology and chemistry of substances with cardiac activity II Effect of 1 ascorbic acid and some related compounds and of hydrogen peroxide on the isolated heart of the frog J Pharmacol and Exper Therap 77 113-122, 1943.
- 133 KRAYER, O AND SCHÜTZ, E Mechanische Leistung und einphasisches Elektrogramm am Herz Lungen Präparat des Hundes. Ztschr f Biol, 92 453-461, 1932
- 134 KROGH, A, LINDBERG, A. L., AND SCHMIDT-NIELSEN, B The exchange of ions between cells and extracellular fluid. II The exchange of potassium and calcium between frog heart muscle and the bathing fluid. Acta physiol Scandnav, 7: 221-237, 1944
- 135 KRÖGER, M Die gleichzeitige Einwirkung von Coffein und Scillaren auf den Sauerstoffverbrauch überlebenden Gewebes. Inaugural dissertation, 23 pp., Universität Münster 1935
- 136 KUFFLER, S. W The relation of electrical potential changes to contracture in skeletal muscle. J. Neurophysiol., 9: 367-377 1946.
- 137 KUFFLER, S. W Membrane changes during excitation and inhibition of the contractile mechanism. Ann N Y Acad. Sci 47 767-779, 1947
- 138 KUTSCHERA AICHBERGEN, H Über Herzschwäche. Wien Arch f inn Med., 18 209-358, 1929
- 139 LASCH, F AND TRIGER, K Experimentelle Untersuchungen über die Beeinflussung des Kohlehydratgehalts im Herzmuskel II Cardiac. Ztschr f d. ges. exper. Med., 83 588-592 1933
- 140 LECOMTE, L Effet Lundsgaard et oxydants Arch internat de physiol, 56 75-88, 1948
- 141 LENOLE, L Digitaliskörper und verwandte herzwirksame Glykoside (Digitaloide) In Heffters Handbnch der experimentellen Pharmakologie Ergänzungswerk, 1.Bd., 11-263 Julius Springer, Berlin, 1935
- 142 LEPPERMANN, B Vergleichende Untersuchungen über den Einfluss von Strophanthin und seines Aglykons Strophanthidin auf den anaeroben Kohlehydratstoffwechsel. Inaugural dissertation, 19 pp., Universität Münster, 1933
- 143 LÉVY, J AND LIBERT, O Pharmacodynamie cellulaire VIII Etude du mode d'action des digitaliques. Respiration des cellules du myocarde sous l'influence des scillairènes A et B Bull Soc. chim. biol., 29 269-273 1947
- 144 LÉVY, J, LIBERT, O AND SCHWOB, A Pharmacodynamie cellulaire. V Etude du mode d'action des digitaliques. Respiration des cellules du myocarde sous l'influence de l'ouabaïne. Bull Soc. chim. biol., 28 647-651 1946.
- 145 LÉVY, J AND SCHWOB, A Pharmacodynamie cellulaire IV Etude du mode d'action des digitaliques Composition d'une solution de Ringer permettant la respiration maximum des cellules du myocarde chez divers animaux rat, cobaye chien Bull Soc chim biol, 28 642-647, 1946
- 146 LIBERT, O Pharmacodynamie cellulaire VI Etude du mode d'action des digitaliques Respiration des cellules du myocarde sous l'influence de la digitaline Bull Soc chim biol, 28 771-778, 1946
- 147 LIBERT, O. Pharmacodynamie cellulaire. VII Etude du mode d'action des digitaliques Métabolism glucidique du myocarde sous l'influence de la digitaline. Bull Soc. chim. biol., 28: 778-782, 1946.
- 148 LIEBIG, H Untersuchungen über den Kohlehydratstoffwechsel des Herzens II Experimentelle Untersuchungen. Der Einfluss der Digitalisgruppe auf den Kohlehydratgehalt des Herzens. Arch f exper Path. u Pharmacol., 196: 137-147, 1940
- 149 LINZEL W Über den Einfluss der Digitaliskörper auf die Methylenblauentfärbung Inaugural dissertation, 16 pp., Universität Münster 1932

150. Lipmann, F Metabolic generation and utilization of phosphate bond energy Adv in Enzymology, 1 92-162, 1941.
151. LOHMANN, K. AND SCHUSTER, P Über das Vorkommen der Adenin Nucleotide in den Geweben. II Mitteilung: Herzmuskulatur Biochem Ztschr 282 104-103 1935
152. LOHMANN, K. AND WEICKER, B Stoffwechsel des Herzens. In Oppenheimer, C Handbuch der Biochemie des Menschen und der Tiere, 2nd Ed., Ergänzungswerk 3: 414-430, 1935
153. LORENTE DE NÓ A study of nerve physiology, Part I Studies from the Rockefeller Institute for Medical Research 131 XVI and 496 pp 1947
154. LUNDEN, G AND STRÖM, G The influence of g strophanthin on hypodynamic and anoxic heart muscle of the frog. Acta physiol. Scandinavica, 15 279-295 1948
155. MALINO, H. M. AND KEATER, O The action of the erythrophleum alkaloids upon the isolated mammalian heart. J Pharmacol. and Exper Therap 86 66-78 1946.
156. MALLOV, S AND ROBB, J B. Behavior of actomyosin threads. Fed. Proc., 8 104-105, 1949
157. MANGUN, G H AND MYERS, V C Cardiac muscle Further studies Investigation of chemical changes in myocardial insufficiency with special reference to adenosinetriphosphate. Arch. Int. Med., 78 441-446 1946
158. MANGUN, G H, REICHEL, H. S., AND MYERS, V C Further studies on human cardiac and voluntary muscle, possible implications of changes in the creatine, phosphorus and potassium content, with special reference to heart disease Arch. Int. Med. 67: 320-332 1941
159. MARDONES J Mecanismo de acción de los glucósidos cardíacos. Rev. med. de Chile, 76 392-399, 1948.
160. MARDONES, J, MUÑOZ, C AND SEPÚLVEDA, M. Acción de los glucósidos digitales sobre intestino delgado aislado de conejo Interferencia de algunas condiciones metabólicas Bol. Soc. Biol. Santiago de Chile 5 10, 1948.
161. MATTONET C Chemischer Beitrag zur Frage der Herzmuskelschädigung durch Thyronin. Ztschr f d. ges exper Med. 90 237-244, 1933.
162. MEL'NIKOVA, V F Vliyaniestrofantina na obmen molodjnoj kisloty v serdce pri eksperimentalnoi nedostatodjnosti (Effect of strophanthin on the exchange of lactic acid in the heart during experimental failure) Bull Ekspier Biol. Med., 13 47-49, 1942.
163. MENDEZ, R. Pharmacology and chemistry of substances with cardiac activity III The effect of simple unsaturated lactones and t-butyl hydrogen peroxide on the isolated frog heart. J Pharmacol. and Exper Therap 81: 151-169, 1944.
164. MENDEZ, R. AND PERALTA, B The action of sulfhydryl reagents upon the isolated frog heart. J Pharmacol. and Exper Therap, 90 128-137, 1947
165. MEYERHOFF, O Über ein neues autoxydables System der Zelle (Die Rolle der Sulfhydrylgruppe als Sauerstoffüberträger) Arch. f d. ges. Physiol., 199 531-556 1923
166. MEYERHOFF O The main chemical phases of the recovery of muscle. Ann. N Y Acad Sci., 47 815-832 1947
167. MOE, G K. Unpublished observations, 1945 Quoted by Krayner O and Acheson, G H (131)
168. MOE, G K. AND VISCHEK, M. V Studies on the native glucosides of digitalis lanata with particular reference to their effects upon cardiac efficiency and their toxicity J Pharmacol. and Exper Therap., 64. 65-85, 1933
169. MOE, G K. AND VISCHEK, M. V The mechanism of failure in the completely isolated heart. Am. J Physiol. 125 461-473 1939
170. MOSES L. E Mechanism of the effect of hyperthyroidism on cardiac glycogen. Am. J Physiol., 142 686-699 1944.
171. MRATZEK, R. G AND REED C I. The effect of muscular fatigue on the solubility of myosin. Am. J Physiol., 149 177-179 1947
172. MOOG, H. Eine Erweiterung der Methode zur Bestimmung der Phosphorfraktionen im Muskelgewebe unter besonderer Berücksichtigung der Phosphagenbestimmung im Warmblüterherzen. Arch. f. exper. Path. u. Pharmacol., 173 333-340 1933
173. MYERS V C Some chemical changes in the myocardium accompanying heart failure. Bull N Y Acad. Med. 18 303-317 1942
174. MYERS, V C AND MANOUN, G H. Comparative studies on creatine, phosphorus and potassium in various muscle tissues. J Biol. Chem., 137- 701-709, 1940.
175. MYERS V C AND MANGUN, G H Some chemical observations on the human heart in health and disease. J Lab Clin. Med., 26 199-210 1940-41
176. NAKAMURA, K. SAUNDERS P. R. WEBB J L., LAWSON, H. C AND THIEYEN, C H Metabolism of the heart in relation to drug action. IV Effects of various substrates upon the isolated perfused rat heart. Am. J Physiol. 158. 269-278 1949
177. NEUBERG C AND SANDBERG, M. Über Stimulatoren der alkoholischen Zuckerspaltung. IX Mitteilung über chemische definierbare Katalysatoren der Gärung. Biochem Ztschr, 126 153-178, 1921-23
178. OCHOA S. Enzymatische Milchsäurebildung in der Herzmuskulatur Biochem. Ztschr, 290- 62-70, 1937
179. OLIVOS, P., PEINA, M. AND MARDONES J Unpublished experiments. Quoted by Mardones, J (159)
180. OLSON R E., PEARSON, O H. MILLER, O N AND STARR F J The effect of vitamin deficiencies upon the metabolism of cardiac muscle in vitro I The effect of thiamine deficiency in rats and ducks. J Biol. Chem. 175 499-501, 1948
181. OLSON R. E AND SCHWARTZ, W B Cardiac muscle metabolism and congestive heart failure. To be published.
182. PARNAS J K. AND OSTERN, P Über die Ammoniakbildung im isolierten Frochherzen. Biochem Ztschr, 234. 307-322 1931
183. PAULUS, H. Über den Einfluss der Glykoide Adonidin und Convallamarin auf die Dehydratierungsvorgänge im Gewebe. Inaugural dissertation 13 pp., Universität Münster, 1933

- 184 PEARSON, O H HASTINGS, A B AND BENTING, H Metabolism of cardiac muscle Utilization of  $C^{14}$  labelled pyruvate and acetate by rat heart slices *Am J Physiol*, 158 251-260, 1949
- 185 PETERS, H AND VISSCHER, M B Energy metabolism of the heart in failure and influence of drugs upon it. *Am Heart J*, 11 273-291, 1936
- 186 PINOTTI, O L'influenza del fegato sul metabolismo energetico del cuore. *Arch di fisiol.*, 42 180-182, 1941
- 187 PATTERSON, S W, PIPER, H AND STARLING, E H The regulation of the heart beat. *J Physiol.*, 45 465-513, 1914
- 188 PLOTKA, C PETERFALVI, M, JEQUIER, R AND VELLUZ, L Action of a new phosphorylation derivative of thiamine on the heart A study of thiamine triphosphoric acid *Am J Physiol*, 158 278-286, 1949
- 189 POHLE, K Zur Frage der herzwirksamen Form des Kalles *Arch f exper Path u Pharmacol.*, 178 107-147, 1935
- 190 POLLACK, H, FLOCK E ESSEN, H E AND BOLLMAN, J L Phosphorus compounds in the perfused heart of the dog *Am J Physiol*, 119 97-101 1934
- 191 PÖTTING K Über den Einfluss von Lanadigin und Strophanthin und ihrer Genine, Lanadigenin und Strophanthidin, auf die Gewebsoxygenationen Inaugural dissertation, 23 pp Universität Münster, 1933
- 192 PÖTZ, H Vergleichende Untersuchungen über den Einfluss von Digitoxin und seines Aglykons Digitoxigenin auf den anaeroben Kohlehydratabbau im Gewebe Inaugural dissertation, Universität Münster, 1933
- 193 RANDELS F S, HEMWICH W A, HORNBERGER, E AND HEMWICH, H E Influence of vitamin B<sub>1</sub> deficiency on pyruvate exchange of heart. *Am Heart J*, 33 341-345 1947
- 194 REIN, H Der Sauerstoffverbrauch des Warmblüterherzens Zentralbl f inn Med 56 707-708 1935
- 195 REIN, H Über die physiologischen Aufgaben des Adrenalins als Kreislaufhormon Verhandl d deutsch. Ges. f Kreislaufforsch., 10 Tag 27-58 1937
- 196 REIN, H Physiologische Beziehungen zwischen der Leber und dem Energiestoffwechsel des Herzens *Klin Wchnschr* 21 573-577, 1942
- 197 ROHDE, E Stoffwechseluntersuchungen am überlebenden Warmblüterherzen I Mitteilung Zur Physiologie des Herzstoffwechsels *Ztschr f physiol Chem* 68 181-235, 1910
- 198 ROHDE E Über den Einfluss der mechanischen Bedingungen auf die Tätigkeit und den Sauerstoffverbrauch des Warmblüterherzens *Arch f exper Path u Pharmacol* 68 401-434, 1912
- 199 ROHDE, E AND OGAWA S Gaswechsel und Tätigkeit des Herzens unter dem Einfluss von Giften und Nervenreizung *Arch f exper Path u Pharmacol* 69 200-238, 1912
- 200 RIESSER, O AND KLEIN, P Pharmakologische Untersuchungen am Musculus rectus des Frosches *Arch f exper Path u Pharmacol* 170 580-591 1933
- 201 ROBB, J S Heart *Ann Rev Physiol*, 11 337-434, 1949
- 202 ROSENHOVEN, H Untersuchungen über den Milchsäurestoffwechsel des isolierten Hundeherzens *Ztschr f d ges exper Med* 90 225-236, 1933
- 203 RÜHL A Über den Gasstoffwechsel am insuffizienten Herzen I Herzinsuffizienz durch Histamin *Arch. f exper Path u Pharmacol*, 172 568-583, 1933
- 204 RÜHL, A Über den Gasstoffwechsel des insuffizienten Herzens II Herzinsuffizienz durch Barbituratsedative und Avertin *Arch f exper Path u Pharmacol*, 174 98-110, 1934
- 205 RÜHL, A Über die Bedeutung der Milchsäure für den Herzstoffwechsel *Klin Wchnschr*, 13 1529-1534, 1934
- 206 RÜHL, A Wesen und Bedeutung der Gasstoffwechseländerungen am insuffizienten Herzen *Arch f exper Path u Pharmacol* 187 22-40 1937
- 207 RÜHL, A AND THADDEA S Anoxie und Milchsäurestoffwechsel von Herz und Lunge *Arch f exper Path. u Pharmacol* 191 452-464, 1939
- 208 RÜHL, A AND WIEHLER A Über die Wirkung des Strophanthins auf den Gasstoffwechsel des insuffizienten Herzens *Arch f exper Path u Pharmacol*, 175 665-680, 1934
- 209 RUPPERT, H Über Bindungs- und Wirkungsbedingungen des sauren Farbstoffs Brillantkongorot am Herzmuskel und den Nachweis von Permeabilitätsveränderungen *Arch f exper Path. u Pharmacol.*, 200 512-526 1943
- 210 SALOMON, K AND RIESSER O Zur Frage des Einflusses von Digitoxin und Strophanthin auf oxydative Vorgänge in Versuchen am Modell sowie am atmenden überlebenden Herzmuskelgewebe. *Arch f exper Path u Pharmacol* 177 450-462, 1935
- 211 SANDOW, A ET AL Muscular contraction *Ann N Y Acad. Sci*, 47 665-930, 1947
- 212 SANZI, R. Die pharmakologische Wirkung neuer Alkaloide, die aus dem Erythrophleum Guineense und aus dem E Coumunga isoliert wurden *Arch f exper Path u Pharmacol*, 193 152-169, 1939
- 213 SCHÄFER, K Über das Zusammenwirken von Coffein und Strophanthin am intermediären Stoffwechsel Inaugural dissertation, 27 pp Universität Münster, 1934
- 214 SCHLEIBINGER, I Der Glykogenabbau im Muskel unter der Einwirkung von Giften aus verschiedenen pharmakologischen Gruppen Inaugural dissertation, 23 pp, Universität Münster, 1933
- 215 SCHMITT, F O The oxygen consumption of stimulated nerve. Cold Spring Harbor Symposia, 4 188-193, 1936
- 216 SCHÖLLER, W AND GERRAE M Über die Beeinflussung der Glykolyse von lebenden Hefezellen I *Biochem. Ztschr*, 172: 358-372, 1928
- 217 SCHUMANN, H Experimentelle Befunde zur Frage gesteigerter Thyroxinempfindlichkeit des mechanisch vermehrt belasteten Herzmuskels *Ztschr f d ges. exper Med*, 165 577-583, 1939
- 217a SCHUMANN, H Untersuchungen über den Muskelstoffwechsel des Herzens *Ergebn d inn Med u Kinderh.*, 62 869-918, 1942
- 218 SIGGE, G Recherche sul meccanismo d'azione dei digitalici *Arch internat. de pharmacodyn et de therap*, 75 227-242, 1948
- 219 SHELLEY W B, CODE, C F AND VISSCHER, M B The influence of thyroid dimorphonol and swimming on

- the glycogen and phosphocreatine level of the rat heart in relation to cardiac hypertrophy *Am J Physiol.*, 135: 632-638, 1942-43
220. SNEROD, T. R. Effects of digitals on electrolytes of heart muscle *Proc. Soc. Exper Biol and Med*, 65 89-92, 1947
221. SINCLAIR, R. G. The physiology of the phospholipids *Physiol. Rev.*, 14. 351-403, 1934
222. SUTTIE, D. H. Oxidation of pyruvate by heart muscle. *Biochem J.*, 34 1046-1058, 1940
223. SOMMERKAMP, H. Die Wirkung von Herzmitteln auf das sauerstofflos schlagende Froschherz *Arch f exper Path u Pharmacol.*, 124. 243-258, 1927
224. SPAULDING, M. E. AND GRAHAM, W. D. The enzymatic degradation of coenzyme and the inhibitory action of  $\alpha$  tocopheryl phosphate *J Biol. Chem.*, 170 711-718, 1947
225. STARLING, E. H. The Lincere lecture on the law of the heart. Given in Cambridge, 1915, 27 pp., Longmans, Green and Co. London, 1918
226. STARLING, E. H. AND VISSCHER, M. B. The regulation of the energy output of the heart. *J Physiol.*, 62 243-261, 1927
227. STRAUB, W. Die Digitalisgruppe. In *Hefters Handbuch der experimentellen Pharmacologie*, 2 Bd., 1355-1452 Julius Springer, Berlin, 1924
228. SZENT-GYÖRGYI, A. Chemistry of Muscular Contraction, IV and 150 pp. Academic Press, Inc., New York, 1947
230. TESTA, C. Influenza del cardiocinetica sui processi di deidrogenazione del muscolo cardiaco. *Rassegna di terap e pat. clin.*, 5 727-735 1933
231. TÖBREN, H. Der Einfluss des Strophanthins auf die Milchsäurebildung. Inaugural dissertation, 20 pp., Universität Münster, 1933
232. VAN LIERE, E. J., CRICKER, G. AND HALL, E. J. The effect of digitals on acute cardiac dilatation produced by anoxemia. *J Pharmacol and Exper Therap*, 52 408-417, 1936
233. VICTOR, J. The effect of ouabain on the oxygen consumption of cardiac ventricular muscle. *Proc. Soc. Exper Biol and Med*, 30 399-402 1932.
234. VISSCHER, M. B. Der mechanische Wirkungsgrad des versagenden Herzens *Arch f exper Path. u Pharmacol.*, 191: 266-274, 1938
235. VISSCHER, M. B. The energy transformation by the heart and the mechanism of experimental heart failure. In *Publication 13 of Am. Assoc. Adv. Sci., Blood, heart and circulation*, 176-183, Washington, 1940
236. VISSCHER, M. B. AND MULLEN, A. G. The carbohydrate metabolism of the heart *Am J Physiol.*, 94 630-640, 1930
237. WASSERMAYER, H. AND ROHRBACH, A. Über chemische und kolloid-chemische Prozesse im Herzmuskel bei der experimentellen Myokarditis *Arch f exper Path. u Pharmacol.*, 166 375-391, 1932
238. WEARN, J. T. Morphological and functional alterations of the coronary circulation. *Bull N Y Acad Med*, 17 754-777, 1941
239. WEDD, A. M. The influence of digoxin on the potassium content of the heart. *J Pharmacol and Exper Therap* 65 268-274, 1939
240. WEDD, A. M., BLAIR, H. A. AND DWYER, G. K. The effect of digoxin on the cold blooded heart and its bearing on the mechanism of digitalis action. *J Pharmacol and Exper Therap* 72 391-403 1941
241. WEISE, H. Digitalis XVII and 290 pp. Georg Thieme Leipzig, 1936
242. WEISE, H. AND WIGAND, C. Zur Begründung der Kombinationstherapie Digitalis Coffein. In *I. G. Farbenindustrie, Medizin und Chemie*, Bd. 2, 143-155, Leverkusen, 1934
243. WEICKER, B. Über den Mechanismus des tätigen Herzmuskels *Arch f exper Path u Pharmacol.*, 174 383-404, 1934.
244. WEICKER, B. Stoffwechselwirkung des Strophanthins am Warmblüterherzen *Arch f exper Path u Pharmacol.*, 178 524-533 1935
245. WEISSICKER, V. FRH V. Einige Beobachtungen über die Verteilung sowie die arbeitsteilgerade Wirkung von Herzglykosiden. *Arch f exper Path u Pharmacol.*, 81 247-250, 1917
- 245a. WERTHEIMER, E. Beitrag zur Frage des Stoffwechsels beim schlagenden Kaltblüterherzen II. Mitteilung *Arch. f d. ges. Physiol.*, 227 100-109 1931
246. WIGGERS, C. J. AND STIMSON, B. Studies on the cardiodynamic actions of drugs. III. The mechanism of cardiac stimulation by digitalis and g-strophanthin. *J Pharmacol and Exper Therap* 30 251-269, 1927
247. WILBRANDT, W. AND KOLLER, H. Die Calciumwirkung am Froschherzen als Funktion des Ionenungleichgewichts zwischen Zellmembran und Umgebung. *Helv Physiol Acta.*, 6 203-221, 1949
248. WILKINS, W. E. AND CULLEN, G. E. Electrolytes in human tissue. III. A comparison of normal hearts with hearts showing congestive heart failure. *J Clin Investigation*, 12 1063-1074, 1933
249. WILLIAMS, W. L. Reaction of digoxin injured myocardium of rats and mice to vital dyes. *Anat Rec.*, 97 99-122, 1947
250. WISE, W. MEYER, J. KATZ, L. N., LENDRUM, B. AND JOCHIM, K. The oxygen consumption and mechanical efficiency of the heart before and during heart failure. *Am. J Physiol* 147 23-37 1946
251. WOLLENBERGER, A. The respiratory and phosphate metabolism of the mammalian heart in experimental failure and following the administration of cardiac glycosides. Doctoral thesis IV and 152 pp. Harvard University, 1946.
252. WOLLENBERGER, A. Influence of cardiac glycosides on respiration and glycolysis of heart muscle and brain. *Fed. Proc.* 6 41 1947
253. WOLLENBERGER, A. Metabolic action of the cardiac glycosides. I. Influence on respiration of heart muscle and cortex. *J Pharmacol and Exper Therap* 91 39-51 1947
254. WOLLENBERGER, A. On the energy rich phosphate supply of the failing heart. *Am J Physiol* 150 733-740 1947

- 255 WOLLENBERGER, A Effect of ouabain and digitoxin on the energy rich phosphate store of the heart. Fed. P 7 266, 1948
- 256 WOLLENBERGER, A Action of narcotics and local anesthetics on the respiration of heart muscle. J Pharm and Exper Therap, 94 444-454, 1948
- 257 WOLLENBERGER, A Respiratory activity in vitro of cardiac muscle of ouabainized dogs. Fed. Proc., 8 348, 19
- 258 WOLLENBERGER, A Metabolic action of the cardiac glycosides II Inhibition of the Pasteur effect in h To be published
- 259 WOLLENBERGER, A Unpublished experiments.
- 260 WOOD, E H AND MOE, G K Electrolyte and water content of the ventricular musculature of the heart-lung preparation with special reference to the effects of cardiac glycosides. Am J Physiol., 136 515-522, 1942
- 261 WOOD, E H AND MOE, G K Blood electrolyte changes in the heart-lung preparation with special reference the effects of cardiac glycosides Am J Physiol, 137 6-21, 1942.
- 262 YAFFE, S J Unpublished experiments
- 263 YORIMITSU T Über den Einfluss verschiedener Herzmittel auf den Kohlehydratstoffwechsel des Herzes Jap J Med Sc 4, Pharmacol 13 Abstracts, 21-22, 1940-41
- 264 YORIMITSU T Über den Kohlehydratstoffwechsel im Herzmuskel, Leber und Skelettmuskel bei Verwendung verschiedener Herzmittel II Kohlehydratstoffwechsel in Leber und Skelettmuskel. Jap J Med. Sc., 4, Pharmacol, 14 Abstracts, 34, 1941
- 265 ZWICKSTER, G H AND BOYD T E Reversible loss of the all or none response in cold blooded hearts treated with excess potassium Am J Physiol, 113: 550-557, 1935



# CERTAIN ASPECTS OF THE PHARMACOLOGY OF THE SALICYLATES\*

PAUL K. SMITH

*Department of Pharmacology, The George Washington University School of Medicine,  
Washington 5, DC*

Few drugs have enjoyed an uninterrupted popularity comparable to that of the salicylates. Introduced as plant extracts centuries ago, the salicylates were among the first drugs to be synthesized and their use has increased enormously with time, the present annual production being approximately 6000 tons in the United States alone (206). Although many salicylates have been introduced into therapy, those most widely employed are acetyl-salicylic acid, sodium salicylate and methyl salicylate. It is fortunate for the present reviewer that a summary of the earlier studies on salicylates was published in 1927 by Hanzlik (126, 127) and more recently a complete bibliography with more than 4000 references was compiled by Gross and Greenberg (113). Accordingly, this review will omit most observations of primarily historical interest, as well as many studies that seem at this time to be of minor importance, and will concentrate on selected features of the pharmacology of the three most widely used salicylates.

**Terminology** The word "salicylate" is generally employed as a generic term for the entire class of compounds containing the salicyl radical  $C_6H_4(OH)COO-$ , and will be used for this purpose, in application to the free acid, its salts, ethers and esters. The anion  $C_6H_4(OH)COO-$  will be called salicylate ion, and its salts will be designated as salicylic salts. Occasionally the term "aspirin" is used instead of acetylsalicylic acid. The term salicyl is sometimes employed to designate the salicylic acid radical.

**Methods of Determination** Early methods for determining concentrations of salicylate in biological fluids usually involved preliminary procedures for concentrating the drug, such as distillation (262) and sometimes bromination of the compound (40), but these require relatively large samples. At present, methods determining the compounds without preceding separation are preferred. The color which develops when a dilute solution of salicylate reacts with ferric ion (43, 98, 175, 262, 272) is still the basis for the most common method employed. Recently a very sensitive fluorophotometric procedure (238) has been described.

## PHYSIOLOGICAL DISPOSITION

**Absorption** The free acid, salts and esters are readily absorbed after cutaneous application (200). Methyl salicylate, which is frequently applied to the skin by rubbing, is so rapidly absorbed that measurable concentrations may be detected in the urine within 15 minutes (26), absorption is increased by dissolving the ester in alcohol, in liquid petrolatum or in anhydrous lanolin (44). Normal

\* Original investigations and other work incidental to the preparation of this review were supported in part by a grant-in-aid of research from the Institute for the Study of Analgesic and Sedative Drugs.

human subjects, to whom 0.2 gm is applied locally, excrete about one fourth of the methyl salicylate in the urine (26). The absorption of various salicylic acid esters is rapid but the rate decreases as the length of the carbon chain of the ester increases (45). Salts of salicylic acid with organic bases may be absorbed three to five times as rapidly as sodium salicylate, when applied to the human skin (123).

Absorption of sodium salicylate from the oral cavity is relatively slow. No measurable concentrations appear in the urine of rabbits for 25 to 56 minutes after the drug is introduced in the mouth (31). In dogs with ligated pylorus, salicylates are fairly rapidly absorbed from the stomach, approximately two thirds of the dose are absorbed within one hour after an aspirin solution of pH 2.5 is administered, and somewhat less when sodium salicylate solution of pH 6.8 (41) is given. The absorption of both salicylic and acetylsalicylic acid from the stomach is slower when the solution is near neutrality than when the pH is low. In comparable experiments, acetylsalicylic acid solution of pH 2.9 is absorbed to the extent of 49 per cent in one hour whereas a salicylic acid solution of pH 2.5 is absorbed to the extent of 64 per cent in one hour. When salicylates are given orally to human subjects, the rate of absorption depends to an appreciable extent upon the emptying time of the stomach. Effervescent mixtures containing salicylate decrease the emptying time of the stomach (177), owing partly to the weak alkalinity of the solution and partly to the carbon dioxide produced. When salicylic acid is perfused through loops of the upper ileum in dogs, approximately two thirds of the amount perfused are absorbed in 30 minutes regardless of whether the solution is introduced at pH 2.5 or at pH 6.8 (41). When acetylsalicylic acid is introduced the absorption is approximately 50 per cent complete in 30 minutes. Absorption is slower in the stomach at high than at low pH values but the difference is less when the solutions are introduced into the intestine. Sodium salicylate, calcium salicylate and free salicylic acid are absorbed from the intestine of dogs more rapidly than acetylsalicylic acid (41). However, even in the case of acetylsalicylic acid, approximately two thirds of the dose are absorbed in one hour. When the absorption technic of Cori was employed in rats, it was found that sodium bicarbonate does not influence the rate of absorption from the gut (275). In studies on dogs designed to eliminate the effect of gastric emptying time and possible effects of anesthesia, solutions of salicylic acid made isotonic with sodium chloride and buffered to various pH values from 2.5 to 7.8 were passed through Thiry-Vella jejunal loops in dogs (171). The absorption rate was greatest at approximately pH 5.5 to 5.8 and approximately half as great at pH 7.8.

In contrast to the rapid absorption from the upper gastrointestinal tract, salicylates are rather slowly absorbed when given rectally, although measurable concentrations may be detected in the urine 15 minutes after administration (136). Some investigators have found that rectal administration of salicylate is feasible in patients (136). Others, using rabbits, have even concluded that absorption is more rapid from the large intestine than from the upper gastrointestinal tract, for example, one hour after the oral administration of 0.25 gram of

salicylate, 60 mgm per cent is found in the blood, compared to 85 mgm per cent after the rectal administration of the same dose (33). In general, however, those who have used rectal administration find low plasma levels, or at least that a given blood level is reached more slowly (143). Sodium bicarbonate diminishes the rate of absorption of salicylate from the rectum of man (212).

When sodium salicylate is given subcutaneously to rabbits the maximum concentration in the blood is attained between 30 and 60 minutes after administration; more than 40 per cent is excreted in six hours (30). After intramuscular administration of sodium salicylate to rabbits appreciable amounts are found in the urine after 30 minutes (32).

In rabbits, measurable concentrations of salicylate appear in the urine 40 minutes after intrapleural and 26 minutes after intraperitoneal administration, maximum urinary levels are obtained approximately 30 minutes after intraperitoneal and approximately 60 minutes after intrapleural administration (34). Intratracheal injection results in rapid absorption, the drug appearing in the urine two to 12 minutes after administration (30). A 2.5 per cent solution of sodium salicylate is not absorbed from the bladder of normal rabbits but is readily absorbed in animals in which the bladder mucosa is inflamed, the rapidity of absorption depending upon the degree of inflammation (32).

The evidence indicates that the salicylates are among the most rapidly absorbed drugs, regardless of the route of administration. Although the rate of absorption depends somewhat upon the pH, it is nevertheless rapid at all hydrogen ion concentrations within the physiological range.

*Plasma Levels* The plasma levels of salicylate are a more precise measure of the amount of drug in the body than are whole blood levels, since the concentrations in the erythrocytes and plasma are not the same (168, 246). After a single oral dose of two grams of sodium salicylate in normal young men, the average peak salicylate level in the plasma is approximately 15 mgm per cent and is reached in 100 to 120 minutes (246). Apparently acetylsalicylic acid is somewhat more slowly absorbed than sodium salicylate, two gram oral doses in normal young adults give peak levels of approximately 10 to 12 mgm per cent after two to four hours (246). The levels attained are approximately proportional to the dose. Doses of 100 mgm of sodium salicylate per kgm give approximately 24 mgm per cent peak concentration in the plasma (212), 150 mgm per kgm give 28 mgm per cent, and 200 mgm per kgm give 40 mgm per cent.

*Distribution in the Body* A large part of the salicylate in the blood is bound to plasma proteins. As long ago as 1923 it was demonstrated that plasma salicylate is not completely diffusible through a semi-permeable membrane (54), and it was concluded from studies on the renal clearance of salicylate that most of it is bound to serum proteins (55). In *in vitro* studies, as much as 50 per cent of sodium salicylate was found to be bound by serum proteins at salicylate concentrations of 200 mgm per cent (102). Most of the salicylate in the sera of rheumatic fever patients is associated with non-diffusible solids of the plasma (246), and at levels of 200 mgm per cent only about 30 mgm per cent is diffusible, at

levels of 400 mgm per cent, only 125 mgm per cent is diffusible, at levels of 500 mgm per cent, about 250 mgm per cent is diffusible Exactly equal amounts of protein-bound salicylate were found in the plasma of patients who had been receiving the drug, and in normal plasma to which comparable amounts of the drug were added It has been suggested that some salicylate is bound to cholesterol (258)

The salicylate content is usually greater in the serum than in the whole blood of animals that have been given sodium salicylate When either single or repeated doses of salicylate are administered orally to rats the concentration of salicylate is usually slightly higher in the serum than in the whole blood (246), but when the concentrations are estimated on the basis of water content, the amount in whole blood is usually slightly higher than that in the serum, which would indicate a slight accumulation of the drug in the erythrocytes The concentration of salicylates in the erythrocytes of normal subjects (168) and of rheumatic fever patients during treatment (246) is quite low and of the same order of magnitude as would be expected if the concentration in the red cells were similar to that found in the plasma ultrafiltrate This suggests that the human red cell membrane is freely permeable to the ultrafiltrable portion of the plasma salicylate and that the drug is not bound by the proteins of the erythrocytes

Early studies (242) demonstrate that the concentrations of salicylate in blood and joint fluid are similar, but the concentration of salicylate in parotid saliva is approximately 0.3 to 0.6 as high as in the serum (169), which suggests that in secretory glands protein binding may influence concentration gradients

Apparently salicylates cross the placental barrier fairly readily When pregnant rabbits are given large doses of sodium salicylate the concentration of salicylate in the fetal serum is approximately two thirds that in the maternal serum (147)

The salicylates are distributed through a volume of body water which appears much greater than that of the extracellular fluid Studies on rats showed that the concentrations in the liver, kidney, and lung are similar to those in the serum (246) When the salicylate concentrations are calculated on the basis of water content, the liver contains about two thirds as much as serum, muscle approximately one fifth as much, the kidneys a similar amount, brain one third to one half as much and lungs contain one half to two thirds as much In the case of a suicide committed with acetylsalicylic acid, the concentration of salicylate in the kidneys was approximately twice that in the liver, that in the spleen and muscle was somewhat smaller, and that in the brain only about half that in the liver (124) The binding of salicylates by proteins other than those of the plasma has not been especially studied, but the above results suggest that they are bound by proteins of most tissues

*The Degradation and Conjugation of Salicylates* It has been known for a long time that a fraction of the administered salicylic acid combines with glycine to form salicyluric acid, a substance analogous to hippuric acid (9, 10) The conjugation product has no therapeutic effect in rheumatic fever patients (253) and

is excreted unchanged after administration to rabbits. In earlier studies in the dog (221) it was found ortho-hydroxyl substituted derivatives of benzoic acid were conjugated with glycine to a smaller extent than the isomeric meta- and para-hydroxyl derivatives, later, the same investigator (223) reported the isolation of salicyluric acid from the urine of men receiving salicylic acid. This suggests that dogs metabolize the drug in a manner different from man, and later observations (246) suggest also that smaller amounts of salicyluric acid are formed in the dog. Rabbits apparently are unable to form salicyluric acid when salicylate is administered (42, 69). Despite the failure of some investigators (125), salicyluric acid can be recovered from the urine of persons who have been given salicylates (9, 10, 11, 12, 138). Holmes found that approximately 60 per cent of the salicylate is excreted in man as salicyluric acid (138).

The thorough studies of Kapp (153) and Kapp and Coburn (154), who found that children excrete 55 to 60 per cent of salicylate as salicyluric acid, leave no doubt that this conjugation product is an important metabolite. Studies on rheumatic fever patients (246) demonstrate that, whereas an appreciable amount of salicyluric acid is excreted in the urine, the amount therein may be no more than one third of the total amount excreted. This is in accord with the observations (153, 176) that rheumatic fever patients apparently metabolize appreciable amounts of salicylic acid in a different manner.

It has been known for a long time that when salicylates are administered to human patients the non-glucose reducing substances of the urine are increased (163). Others measured this increase in reducing substances and ascribed it to glucuronic acid (101). The glucuronic acid appearing in the urine after oral acetylsalicylic acid has been measured quantitatively (193). Some salicylate is excreted with one mole of glucuronic acid and some with two moles (101). In dogs, salicylate is bound to twice its molecular equivalent of glucuronic acid (222). In rabbits, small amounts of both ester glucuronide and ether glucuronide are excreted (42). There is evidence (153) that two different glucuronates appear in human urine, although they have not been isolated in pure form. The amount of salicylate excreted as glucuronate is about 20 to 25 per cent of the total in nonfebrile rheumatic fever patients (246), but the percentage may be smaller in febrile patients (153), partly because a greater amount is metabolized by oxidation.

There is at least one other well-established metabolite of salicylate. It is gentisic acid or 2,5-dihydroxybenzoic acid (6, 9). This substance can be identified by its transient blue color reaction with iron salts in dilute solution. It has been isolated from the urine of rats after the administration of sodium salicylate, acetylsalicylic acid and methyl salicylate (183). It fails to be formed in animals whose livers have been damaged by the administration of phosphorus or carbon tetrachloride (183). Normally from four to eight per cent of the salicylate administered is converted to gentisic acid and related compounds in non-febrile patients, but appreciably more is converted in rheumatic fever patients (153).

Baldoni's isolation (9) of a compound of the general formula  $C_{15}H_{15}NO_8$ , and named "acidoduraminsalicilico", has been verified by Kapp (153). It is closely

related to gentisic acid and may be a combination of gentisic acid and salicylic acid, having the formula  $\text{HO C}_6\text{H}_4 \text{ CO N}(\text{CH}_2\text{COOH}) \text{ C}_6\text{H}_2(\text{OH})_2 \text{ COOH}$

In studies designed to determine the possible precursors of vitamin C, Longenecker *et al* (178) administered acetylsalicylic acid to rats but were unable to find any marked increase in vitamin C excretion and concluded that the salicylate is probably not utilized for ascorbic acid formation

When methyl salicylate is administered to patients, some of it is probably absorbed unchanged Normal subjects eliminate 45 to 57 per cent of it as salicyl, but rheumatic fever patients excrete a more variable percentage (130) In dogs and cats the urine contains much less salicyl after the administration of methyl salicylate than after the administration of sodium salicylate (133) The metabolism of methyl salicylate has not been studied as thoroughly as that of salicylic acid, but its excretion may extend over a longer period than that of sodium salicylate (128)

The metabolism of acetylsalicylic acid differs somewhat from that of sodium salicylate Some of it appears to be absorbed unchanged, since unhydrolyzed acetylsalicylic acid has been found in the plasma of human subjects two hours after they received the drug (168) When isolated intestinal loops of dogs are perfused with acetylsalicylic acid solution, the outflowing solution contains no more free salicylate than the solution introduced, which suggests that the ester is not hydrolyzed in the intestinal lumen but only during or after absorption (171) Hanzlik and Presko (129) found unchanged acetylsalicylic acid in the urine of patients given the drug but their analyses depended upon the difference in intensity of the color reaction with iron before and after hydrolysis of the urine It is known from later work (153, 246) that the glucuronates are hydrolyzed by the preparatory procedures they employed Therefore, their conclusion that 25 per cent of the salicylate was unchanged acetylsalicylic acid is probably erroneous The metabolites are almost equal in amount after administration to human subjects of equal molar amounts of sodium salicylate and of acetylsalicylic acid (246) Normal human plasma hydrolyzed acetylsalicylic acid fairly readily, a dilute solution being almost completely hydrolyzed within three hours (247) The drug is rapidly hydrolyzed to salicylic acid, when administered intravenously to dogs (246), and the tissues of several species of animals are capable of hydrolyzing it readily (247, 265) The liver and kidney of guinea pigs and rats are richest in the hydrolyzing enzyme, each of these tissues containing at least five times as much per gram as the plasma (247, 265), guinea pig brain contains less and muscle none (265) The crude enzyme is a very water-soluble substance not precipitable even by saturated sodium sulfate solution It has its maximum activity at pH 6.0 to 6.5, is readily destroyed by heat, does not hydrolyze acetamide linkages such as in acetyl-para-aminobenzoic acid or acetanilid, but rapidly hydrolyzes ethyl butyrate (247) By concentrates of a crude enzyme prepared by procedures described for liver esterase, both ethyl butyrate and acetylsalicylic acid are hydrolyzed at rates proportional to those observed with the crude tissue extract This indicates that the enzyme is similar to liver esterase (8, 90)

After administration of either sodium salicylate or acetylsalicylic acid, the

serum contains salicylate ion, and after administration of acetylsalicylic acid it also contains a small amount of the unchanged ester (168), but no other metabolite of the two drugs has ever been demonstrated in the serum. The studies of Brodie *et al* (43) show that the plasma contains no salicyluric acid.

**Excretion** Although a small part of administered salicylate may be excreted in other body fluids (156), most of it is excreted in the urine. The quantity as well as the form in which salicylates are excreted varies with the complex of factors which govern the formation of degradation and conjugation products in the tissues and the excretion of the absorbed substances and their metabolites. Of the total dose administered, about 80 per cent is excreted in normal human subjects in the form of compounds containing the salicyl group, and about four to eight per cent is converted to gentisic acid and related compounds (154, 246).

It has been known for a long time that patients tolerate salicylates better when sodium bicarbonate is given simultaneously (264), sodium bicarbonate lessens the tendency to nausea and vomiting. This beneficial effect of sodium bicarbonate has often been attributed to reduction of a local irritant effect in the gastrointestinal tract, but the origin of the late nausea and vomiting is largely central and many observations show that the main effect of sodium bicarbonate is an increased salicylate excretion. Smull *et al* (248) were the first to point out that the administration of sodium bicarbonate definitely lowers the serum salicylate level during the therapy of patients with acute rheumatic fever. They ascribe this reduction to one or more of the following factors: interference with absorption, increased extracellular fluid volume, and increased renal excretion. It was later demonstrated that the last factor is of primary importance. When patients are given sodium bicarbonate in addition to their usual dose of sodium salicylate, the plasma levels promptly fall by 33 per cent, and the amount of free salicylate in the urine increases rapidly and almost in proportion to the diminution of plasma level (246). Moreover, when patients with a fairly constant plasma level of about 45 mgm per cent receive the same dose of salicylate with an equal amount of sodium bicarbonate, the average plasma level falls rapidly to 20 mgm per cent (246). As soon as sodium bicarbonate is discontinued the plasma salicylate concentration rises rapidly to the prior level.

Sodium bicarbonate increases the urinary excretion of salicylate in children so rapidly that the blood level four hours after its administration may be only half of that attained without sodium bicarbonate (187). It has been suggested (53) that alkali should not be given with salicylate except when it is desirable to reduce the plasma level. In dogs, sodium bicarbonate decreases the serum level and increases the output in the urine (275). In both dogs and human subjects, the administration of either sodium bicarbonate or potassium citrate diminishes the plasma level by 10 to 25 per cent by increasing the amount of free salicylate in the urine (212). Ammonium chloride and para-aminobenzoic acid produce the opposite effect. When the urinary pH is increased from 5.2 to 8.0, the free salicylate excreted increases from 10 per cent to about 60 per cent of the total excreted.

The renal clearance of salicylate showed a close relationship to the urinary

related to gentisic acid and may be a combination of gentisic acid and salicylic acid, having the formula  $\text{HO C}_6\text{H}_4 \text{ CO N}(\text{CH}_2\text{COOH}) \text{ C}_6\text{H}_2(\text{OH})_2 \text{ COOH}$

In studies designed to determine the possible precursors of vitamin C, Longenecker *et al* (178) administered acetylsalicylic acid to rats but were unable to find any marked increase in vitamin C excretion and concluded that the salicylate is probably not utilized for ascorbic acid formation

When methyl salicylate is administered to patients, some of it is probably absorbed unchanged. Normal subjects eliminate 45 to 57 per cent of it as salicyl, but rheumatic fever patients excrete a more variable percentage (130). In dogs and cats the urine contains much less salicyl after the administration of methyl salicylate than after the administration of sodium salicylate (133). The metabolism of methyl salicylate has not been studied as thoroughly as that of salicylic acid, but its excretion may extend over a longer period than that of sodium salicylate (128).

The metabolism of acetylsalicylic acid differs somewhat from that of sodium salicylate. Some of it appears to be absorbed unchanged, since unhydrolyzed acetylsalicylic acid has been found in the plasma of human subjects two hours after they received the drug (168). When isolated intestinal loops of dogs are perfused with acetylsalicylic acid solution, the outflowing solution contains no more free salicylate than the solution introduced, which suggests that the ester is not hydrolyzed in the intestinal lumen but only during or after absorption (171). Hanzlik and Presko (129) found unchanged acetylsalicylic acid in the urine of patients given the drug but their analyses depended upon the difference in intensity of the color reaction with iron before and after hydrolysis of the urine. It is known from later work (153, 246) that the glucuronates are hydrolyzed by the preparatory procedures they employed. Therefore, their conclusion that 25 per cent of the salicylate was unchanged acetylsalicylic acid is probably erroneous. The metabolites are almost equal in amount after administration to human subjects of equal molar amounts of sodium salicylate and of acetylsalicylic acid (246). Normal human plasma hydrolyzes acetylsalicylic acid fairly readily, a dilute solution being almost completely hydrolyzed within three hours (247). The drug is rapidly hydrolyzed to salicylic acid, when administered intravenously to dogs (246), and the tissues of several species of animals are capable of hydrolyzing it readily (247, 265). The liver and kidney of guinea pigs and rats are richest in the hydrolyzing enzyme, each of these tissues containing at least five times as much per gram as the plasma (247, 265), guinea pig brain contains less and muscle none (265). The crude enzyme is a very water-soluble substance not precipitable even by saturated sodium sulfate solution. It has its maximum activity at pH 6.0 to 6.5, is readily destroyed by heat, does not hydrolyze acetamide linkages such as in acetyl-para-aminobenzoic acid or acetanilid, but rapidly hydrolyzes ethyl butyrate (247). By concentrates of a crude enzyme prepared by procedures described for liver esterase, both ethyl butyrate and acetylsalicylic acid are hydrolyzed at rates proportional to those observed with the crude tissue extract. This indicates that the enzyme is similar to liver esterase (8, 90).

After administration of either sodium salicylate or acetylsalicylic acid, the



## PHARMACOLOGICAL ACTIONS

*Effects on the Gastrointestinal Tract* Whether salicylates severely irritate the mucous membranes of the enteric tract is controversial. In some cases of poisoning, the gastrointestinal tract showed hemorrhage and ulceration (28), but in other cases of severe poisoning gastrointestinal alterations were absent (82). Even in methyl salicylate poisoning the gastrointestinal symptoms may persist for some time with no permanent damage to the mucosa (219). In dogs, it was possible to produce petechial hemorrhages of the gastric mucosa by intravenous administration of sodium salicylate, oral ingestion of acetylsalicylic acid or subcutaneous administration of methyl salicylate (76). The doses necessary are of the order of 300 mgm per kgm per day for three days or more. Gastric ulceration in rats can be produced by the oral ingestion of 300 mgm of acetylsalicylic acid for 10 days (17). It can also be produced by subcutaneous administration. Patients receiving acetylsalicylic acid for some time gave no evidence of hyperemia or hemorrhage upon gastroscopic examination. Chronic doses produced no gastritis, the epigastric distress observed was ascribed to increased acid production or to pylorospasm (215).

In contrast to the observations on the irritant effects of salicylate on the gastrointestinal tract, it was shown that the administration of aspirin to rats inhibits gastric ulceration. This occurred whether the drug was given intraperitoneally, intravenously or subcutaneously. Aspirin was slightly less effective than sodium salicylate (214). Sodium salicylate in a concentration of 1:2500 does not alter the response of rabbit duodenal or uterine muscle to autonomic drugs (261).

Aspirin given in doses of 70 to 90 mgm per kgm daily for five days to Copepouch dogs caused no increase in gastric secretion but twice this dose was effective (57).

*Effects on the Liver* Liver damage does not occur frequently even after large salicylate doses. However, in several cases of salicylate poisoning hepatic abnormalities of various degrees have been described (56, 82). A 26-year old woman who took 29 grams of acetylsalicylic acid exhibited hepatic damage but no gastrointestinal symptoms (82). In a fatal case of acetylsalicylic acid poisoning (159) in a 54-year old man who probably took 50 to 66 grams of the drug, the autopsy disclosed petechial hemorrhages in the liver. In children, swelling and fatty degeneration of the liver from methyl salicylate have repeatedly been described (81, 86, 162). Similarly, fatty degeneration of the liver in rabbits has been produced experimentally with sodium salicylate (186). Definite liver damage occurs in rats given 400 mgm of sodium salicylate per kgm per day, but in dogs 300 mgm per kgm are not enough to produce significant bromsulphthalein retention (18). *In vitro* studies have demonstrated that high concentrations of salicylate are necessary to depress rat liver respiration (182). In rabbits receiving large doses of sodium salicylate (147) liver glycogen may be greatly diminished. Sodium salicylate had no constant effect upon the nitrogen metabolism of dogs (241), and the excretion of bile salts was not affected (250).

pH The relationship is not linear, the clearance rises slowly with an increase in the urinary pH from 5.5 to 7.0 and rapidly with further increasing pH, at pH 7.7 clearance is approximately 10 times that at pH 6.0 (246)

Perhaps the rise in renal clearance with increase in urinary pH can be explained by considering that the ratio of non-ionized to ionized salicylate increases with the hydrogen ion concentration. The ionized form is highly water-soluble and relatively insoluble in lipids, the reverse is true for the undissociated acid (65). If the renal tubules are more permeable to the lipid-soluble form, then reabsorption of salicylate would be more complete at a low urinary pH. Conversely, at a high pH, the preponderance of the lipid-soluble form would result in high clearance values. If this hypothesis is true then the change in clearance with pH should be independent of the nature of the alkalinizing agent and should also be observed in the excretion of other weak acids. Additional evidence comes from the studies on the excretion of nicotine (121) and of quinacrine in man (150). In both cases these basic substances are excreted much less rapidly when the urine is alkaline, that is, the excretion rate varies with urinary pH in a manner opposite to that of salicylate. Similar conditions have been shown to govern the permeation of weak electrolytes through the cornea (65). In these studies it was shown that permeability is a function of pK. Regardless of whether the active principle is a weak acid such as salicylic acid, or a weak base such as atropine, penetration of the salt is slower than that of the free lipid-soluble principle.

Oral administration of para-aminobenzoic acid has been shown to cause an increase in the plasma level of salicylates (78). This has been amply confirmed by investigators who found that the salicylate levels can be increased two to five times by the administration of two to 24 grams of para-aminobenzoic acid per day (23, 203). In a preliminary report (78) it was not clear whether the para-aminobenzoic acid was administered as the free acid or as the sodium salt, but from a later report (237) it is evident that, in man, in either case the plasma levels are higher. The investigators suggested that para-aminobenzoic acid depresses the formation of salicyluric acid, that the pH of the urine is lower and that both these factors decrease the amount excreted as free salicylate. It was also shown that para-aminobenzoic acid depresses the formation of hippuric acid in man. It has been demonstrated that para-aminohippuric acid is synthesized by the liver (66). This observation coupled with the observation that para-aminobenzoic acid interferes with the formation of salicyluric acid suggests that the enzyme system responsible for the conjugation of the two acids with glycine may be the same.

It seems probable that, in addition to the depression of salicyluric acid formation and the lowering of the urinary pH, para-aminobenzoic acid depresses the excretion of salicylate by depleting the body of base. Para-aminobenzoic acid when given as the free acid is rapidly conjugated to para-aminohippuric acid (245) which is then excreted by the renal tubules (27), probably taking precedence over the excretion of free salicylate or salicyluric acid.

of normal men ingesting four grams of aspirin a day for seven days showed no evidence of a prothrombin deficiency (79)

In summarizing the evidence Quick (224) expresses the opinion that the salicylates resemble dicumarol in their action on prothrombin but are less potent. Agreement exists that salicylates produce a moderate decrease in prothrombin but the doses required are usually large. Even then the tendency to spontaneous hemorrhage is not marked.

Sodium gentisate, a metabolic product of salicylate, when administered to patients in doses up to 10 grams per day, produces no significant increase in prothrombin time (196)

*Effect on Sedimentation Rate* In *in vitro* experiments the sedimentation rate, especially when high, is markedly reduced by sodium salicylate (139). Sodium benzoate and sodium bicarbonate are ineffective. *In vivo* salicylates also decrease the sedimentation rate (140). Plasma fibrinogen is reduced as much as 50 per cent and the reduction is proportional to the total dose given and not to the plasma salicylate level, the sedimentation rates parallel the fibrinogen concentration (226). Patients with rheumatic fever and with carcinoma show similar changes in sedimentation rate and plasma fibrinogen. The alteration of the fibrinogen content of plasma by salicylates (140) is attributed to a direct action both on the plasma and on liver function (226). If salicylates are stopped as soon as the elevated sedimentation rate becomes normal a secondary rise may be observed later (170). Since changes in sedimentation rate are believed to be associated with the activity of the rheumatic fever process, the effect of salicylates on the sedimentation rate cannot be differentiated clearly from the effects of salicylates on the rheumatic process itself.

*Effects on Uric Acid Excretion* In two normal men given 6.6 grams of salicylate per day, excretion of nitrogen, inorganic phosphorus and uric acid was increased (74). It has been concluded that salicylates lower the renal threshold for uric acid (73), when the urinary uric acid concentration rises, the blood level falls (95). Whereas salicylates increase the uric acid in the urine of human subjects (232), the meta and para isomers have no influence. Recent studies (99) of the effects of sodium salicylate upon the excretion of uric acid in the rat show that, at first, the urinary uric acid is increased without a change in the blood uric acid in the renal blood flow or in the glomerular filtration rate, but that with the administration of more salicylate the blood level of uric acid falls. The urinary excretion of uric acid decreases after salicylate administration is discontinued (233). It is concluded that salicylates interfere with the tubular reabsorption of uric acid (99, 103). Earlier studies in the Dalmatian coach hound (111) suggested that salicylates increase uric acid excretion in the urine but decrease it after denervation of the kidneys. This was attributed to alteration of tubular reabsorption. More recently observations indicate that sodium salicylate has no effect on the clearance of uric acid in the Dalmatian dog (100). Gentisic acid in the urine will interfere seriously with the usual determination of uric acid (284).

*Effect on Ascorbic Acid Excretion* Hemorrhages of small vessels attributed to

*Relation to Prothrombin Concentration* Since prothrombin is formed primarily in the liver and since salicylates can induce hypoprothrombinemia, the question of the effect of salicylates on this function of the liver has engaged a great deal of attention. It has been suggested (227) that the prolongation in prothrombin time sometimes observed after salicylate reflects liver damage. It has been postulated (151) that salicylates are converted to dicumarol or some similar substance by bacterial action in the intestinal tract, since oral administration of sodium salicylate to rabbits prolongs the prothrombin time but intravenous administration does not. After oral administration of sodium sulfasuvidine to rabbits salicylates do not prolong the prothrombin time, a fact which suggests that bacteria are responsible for the conversion. Hypoprothrombinemia can be produced in suckling rats (93) by feeding dicumarol or large doses of acetylsalicylic acid to the mothers. The hypoprothrombinemia is less marked in the young if the mother has been given vitamin K (172). Some investigators have found that sodium salicylate, acetylsalicylic acid and methyl salicylate all cause a decrease in the prothrombin in rats (58), but it is also produced by certain other drugs such as acetanilid and antipyrine. Others found no appreciable change in prothrombin time and no dicumarol in the urine after the administration of salicylates to rats (167). The prothrombin levels may be 10 to 60 per cent below normal in acute rheumatic fever (211) at salicylate levels of 35 mgm per cent, but these cases showed no liver damage and the prothrombin time returned to normal despite continued salicylate therapy. Of 24 patients receiving salicylate for 15 to 35 days, prothrombin time was prolonged in six cases by the second to fifth day, but it was always normal by the ninth day (110). A fatal case of sodium salicylate poisoning exhibited a severe hypoprothrombinemia but insignificant hemorrhages (59). Children with rheumatic fever who are on salicylate therapy may develop hypoprothrombinemia but no hemorrhage (92). Although the prothrombin level may return to normal spontaneously, vitamin K is effective in hastening the return. It has been suggested that one mgm of vitamin K be given per gram of salicylate (243) if liver function is adequate, but others believe its use is unwarranted (143). Much larger doses of vitamin K would be required in case of liver damage.

In a rather large group of patients, sodium salicylate produced a moderate but definite decrease in the prothrombin level, unrelated to the dose, no hemorrhagic tendency was observed (49). Others (67) correlate low prothrombin levels with high plasma salicylate levels but find only insignificant hypoprothrombinemia at plasma salicylate concentrations of 35 to 60 mgm per cent. Patients receiving 1.3 to 5.3 grams of sodium salicylate or aspirin a day consistently develop low prothrombin values and exhibit prolonged clotting time (49). In rheumatic fever studies in military personnel (49) the prothrombin level was low in the early stages of treatment but it soon rose. The usual dose in these studies was 10 grams of acetylsalicylic acid per day combined with eight grams of sodium bicarbonate. The prothrombin level after salicylates varies in different species depending on the ease with which vitamin K deficiency can be produced (210). The electrophoretic patterns of the plasma of patients receiving salicylates and

*Analgetic Action* The most important therapeutic action of the salicylates is to reduce pain of moderate severity, but it is difficult to obtain unequivocal evidence of this action either in animals or normal human subjects. When a light is focussed on the tail to produce a pain stimulus, no analgetic effect can be detected in rats even after doses ranging from 0.5 to 2.5 gm. per kgm. (87). Similarly, the threshold to electrical stimulation in mice (160) is not altered by aspirin in doses up to 500 mgm. per kgm. In guinea pigs, acetylsalicylic acid in juxta-lethal dosage abolishes the skin twitch response to pain, but the dose required is 270 mgm. per kgm. (278). Dogs given two grams of aspirin exhibit some analgesia when a twitch of the musculature of the back is taken as an indicator of pain produced by a locally applied stimulus, but the rise in threshold is smaller and of shorter duration than in man (4).

The most common method used for producing pain in human studies of analgetic drugs is that of focussing radiant energy from a bright light source on a blackened area of the skin (134). The heat intensity required to elicit pain is used to measure analgesia. In three trained subjects 1.8 gram of aspirin was reported to raise the pain threshold by 35 per cent (134). The peak rise in threshold is proportional to the dose and the onset of action occurs ten to 15 minutes after ingestion. The effect persists for at least five hours with doses of 0.6 to 1.8 gram. A maximum analgetic effect is maintained with doses of 0.3 gram every two hours but not with 0.6 gram every three hours. Single doses larger than 0.3 gram do not further increase the pain threshold but their effect lasts longer.

These studies have been criticized because of the paucity of subjects and because placebos were not employed (113), also elevation of the pain threshold probably is not an adequate measure of analgetic action (106). Moreover, the sharp twinge of pain produced by superficially applied radiant heat is scarcely comparable to the deep, dull persistent type of pain for which the salicylates are so commonly employed. Much of the rise in pain threshold attributed to salicylates has been ascribed to psychological factors such as suggestion (106).

When pain is induced in human subjects (107) by an alternating current applied to metal fillings in the teeth, aspirin does not elevate significantly the pain threshold (108, 213). When intense pain of an aching character is produced by the aid of a sphygmomanometer cuff, the threshold for pain induced by the alternating current is raised (106, 107), this rise does not occur if aspirin, 0.65 gram, is administered 30 to 40 minutes prior to the test (108, 213).

*Nausea and Vomiting* Since the work of Eggleston and Hatcher (85) in 1912 evidence has been increasing that the salicylates produce emesis by central stimulation. They found that the minimal emetic dose of sodium salicylate in dogs is 200 to 300 mgm. per kgm. when the drug is given intravenously, but 750 mgm. per kgm. when it is administered orally. They obtained evidence of emetic effect even in eviscerated dogs. In rheumatic fever patients in whom high plasma levels of salicylate are maintained, nausea is common as a side effect (51, 52), but nausea is almost as frequent after intravenous as after oral administration of the drug (267) and occurs even when the gastric contents contain no salicylate (57, 112). Nausea is said to occur at salicylate plasma levels of about 37 mgm. per

salicylates have been related to a deficiency of vitamin C (229). The scorbutic guinea pig with bacterial infection exhibits a pathological picture resembling that of rheumatic fever. In earlier studies it was found that administration of acetylsalicylic acid (71) or of sodium salicylate (156) increased the excretion of substances reacting with the indophenol reagent used for the detection of vitamin C. Later it turned out that the reagent reacts with many other reducing substances and, thus, may react with some other component in the urine, formed as a result of the administration of salicylate. By the use of more specific analytical methods, it was found that daily administration of acetylsalicylic acid to normal human subjects in doses varying from 0.6 to 2.6 grams did not affect the excretion of ascorbic acid in the urine (266, 282).

*Effects on the Central Nervous System.* Cases of salicylate intoxication often exhibit evidence of central nervous system disturbances such as delirium (189), and occasionally a psychosis may develop (38). In rheumatic fever patients receiving large doses of salicylates, evidence of mental confusion and dizziness is fairly common (68), and children receiving large doses often exhibit confusion and irritability. Occasionally delirium occurs in rheumatic fever patients after the administration of sodium salicylate (68), but possibly only after intravenous and not after oral administration (189). Cerebral irritability, dullness, stupor and coma may slowly develop after the administration of methyl salicylate (219). Evidence of alterations in the brain has been found after poisoning with sodium salicylate (7). An infant dying from methyl salicylate poisoning exhibited marked hyperemia of the brain and meninges (142).

*Antipyretic Action.* One of the most characteristic actions of salicylate is the fall in body temperature which it produces in animals with fever. This is striking in patients with rheumatic fever (13, 14, 61). In monkeys with fever produced by the subcutaneous administration of yeast, aspirin reduces the temperature, principally by causing increased sweating and vasodilatation (117), in contrast, the drug causes only a slight decrease in the temperature of normal monkeys. In both patients and monkeys, sodium salicylate prevents the rise in temperature produced by the administration of xanthine or caffeine (190). Barbour (15) conducted extensive studies on the mechanism of antipyresis by salicylate and showed that in fever the heat loss increases from a basic value of 37.7 to 52.1 Cal per sq meter. In normal individuals one gram doses of aspirin produce an increase in carbon dioxide output, and heat production rises from 37.8 to 40.3 Cal per sq meter (16). The maximum effect occurs 90 to 120 minutes after drug administration. Respiratory quotient and pulse rate are unchanged. Lesions in the anterior and anterolateral hypothalamus of monkeys cause marked temperature lability but no changes in response to acetylsalicylic acid (118). Barbour (15), as evidence for his idea that antipyretics act by causing hydremia, reported that aspirin was active in this manner, but it was not clear whether a central mechanism was involved. Large doses of salicylates raise the body temperature of dogs (39, 194). The rise is not prevented by nicotine, curare, atropine or decapitation and is therefore peripheral. It is prevented or lessened by potassium cyanide.

predominates in anesthetized animals and the central stimulatory effect in decerebrate animals. In rabbits stimulation of respiration is obtained by salicylate administered intravenously (112). This effect is immediate and the carbon dioxide capacity changes only secondarily. The salicylate is equally effective when given intravenously with sodium bicarbonate (112). The respiratory effect is not depressed by morphine or phenobarbital. In cats and rabbits the respiratory stimulation is not abolished by removal of the carotid bodies or by atropine (112). Bilateral vagotomy invariably alters the respiratory rate and subsequent injections of salicylate fail to stimulate respiration significantly. It is concluded that the mechanism of stimulation is reflex via vagal afferent nerve fibers (112).

*Effect on Acid-Base Balance* The changes in electrolyte pattern are probably related to respiratory stimulation. In almost every case in which it has been measured, the carbon dioxide combining power was found reduced, and the carbon dioxide dissociation curve was of the hypocapnic type owing either to overproduction of acid or to respiratory stimulation (201). The values reported for the carbon dioxide content of the venous blood range between 14 and 38 volumes per cent (7, 76, 209). A value of 57 volumes per cent, well within the normal range, was reported in one case of methyl salicylate poisoning, (86), and in other cases a moderate average fall of 12 volumes per cent has been observed (92). The carbon dioxide content of arterial blood has been found to be as low as 15 volumes per cent (236). In some cases of sodium salicylate or acetylsalicylic acid poisoning the carbon dioxide combining capacity may be 50 volumes per cent (37), but it may later fall to 29 volumes per cent (276). Other investigators, however, have found no correlation between the salicylate level of the plasma and the alkali reserve (110).

Evidence that the diminished carbon dioxide capacity and the rise in pH are due to respiratory stimulation with a subsequent loss of carbon dioxide has been presented by several investigators (3, 68, 91, 137, 208). Normal subjects receiving 12 grams of aspirin exhibit an average rise of 0.06 in blood pH and a fall in plasma bicarbonate of 3.0 millimoles per liter (91). This is associated with an average increase of four liters per minute in respiratory volume. In 27 patients receiving large doses of salicylate the carbon dioxide capacity was decreased to as little as 32 volumes per cent, but the average pH rose from 7.42 to 7.51 (208). Andersen *et al.* (3) studied 7 patients who had received 19 to 32 grams of acetylsalicylic acid in two days and noted a reduction of 20 to 30 per cent in alkali reserve. Similar results were obtained with sodium salicylate but larger doses were believed necessary. The investigators estimated that if hydrochloric acid instead of acetylsalicylic acid were given it would require three to four times as much to produce a comparable fall in alkali reserve. They believe that the hyperventilation is primarily due to central stimulation and that the favorable effect of sodium bicarbonate is probably due to the increased urinary excretion of salicylate.

In advanced salicylate intoxication, particularly in children, both serum carbon dioxide content and pH may be distinctly decreased, but this is usually preceded by a ketosis and follows an initial period of hyperventilation and uncompensated respiratory alkalosis (88).

cent regardless of whether the drug is given orally or intravenously. However, some workers find that the vomiting is not necessarily related to the plasma level, and Graham and Parker (112) report that, although vomiting occurs at an average plasma salicylate concentration of 28 mgm per cent, there is some evidence that local irritation is a factor. In children with rheumatic fever vomiting is not necessarily related to the blood salicylate level, and therefore may be a local effect of the drug (188).

*Effect on Respiration.* Agreement exists that the increased respiratory rate observed after large doses of salicylate is the result of a direct central stimulating effect of the drug. The evidence is increasing that an acidosis, if it occurs, is secondary to the renal adjustment to the respiratory changes (3, 20, 68, 91). In most cases of salicylate poisoning that have been reported, respiration is deep and rapid and the rate may be as high as 38 per minute (21, 81, 91, 201). Occasionally the hyperpnea is of the Kussmaul type and difficult to distinguish from that in diabetic acidosis (37). After methyl salicylate poisoning it is common to find hyperpnea and dyspnea (86, 219) and a marked increase in respiratory rate (162). In normal human subjects 12 grams of acetylsalicylic acid given over a period of eight hours produce a respiratory alkalosis with an increase in respiratory volume of four liters per minute (91). There is frequently an alkalosis with a rise in blood pH, and numbness and tingling of the extremities similar to that observed in tetany (68). Some believe that in patients there is primarily an acidosis which should be treated with sodium bicarbonate (76, 209, 219). Others find a primary hyperventilation due to central stimulation, which then leads to a carbon dioxide-deficit type of alkalosis with an alkaline urine and a low blood bicarbonate (3, 37, 68, 119, 120, 137, 208, 225). Later a ketosis develops with a bicarbonate-deficit type of acidosis (137). The ketosis probably is not related directly to the administration of salicylate since it occurs after voluntary hyperventilation (218) and may result from the administration of alkali (185). It may be related to the inability to synthesize glycogen from protein (185).

Severe dyspnea occurs in patients at an average plasma salicylate level of 50 mgm per cent. Graham and Parker reported that all patients exhibit hyperventilation and some exhibit severe dyspnea when the plasma levels are above 35 mgm per cent (112).

Experimental studies on dogs (39) show that the intravenous administration of 0.19 to 0.6 gram of sodium salicylate per kgm causes hyperventilation. At first, the blood carbon dioxide content, oxygen content or red cell volume of arterial blood are not significantly changed. Later body temperature rises sharply, respiratory rate increases, arterial carbon dioxide falls, and blood pH and hematocrit values increase. Others (119) have confirmed that in dogs there is central respiratory stimulation, in rabbits and cats there is marked respiratory stimulation with increased pulmonary ventilation (152). The intravenous administration of salicylates in dogs and guinea pigs produces an acceleration of the respiratory rate (174). Wright (281), in studies on cats, found that salicylate stimulates both the respiratory center and the sino-aortic nerve endings. Thus the locus of action is both central and peripheral. The peripheral reflex effect



specific, with no cross-sensitivity to salicylic acid. Sensitivity is more frequent in females and in patients with other allergies, especially asthmatics

*Effects on Enzyme Systems* High concentrations of salicylate are necessary to depress the respiration of rat liver *in vitro* (182) They produce some effect on xanthine oxidase, on carboxylase and the dismutation between hexosediphosphate and pyruvate (182) The salicylates inhibit catalase activity (277) and this has been suggested as the mechanism by which antipyretic drugs lower body temperature However, other analgesics such as acetanilid, acetophenetidin, antipyrine and quinine do not inhibit catalase activity It has been demonstrated *in vitro* that sodium salicylate partially inhibits the digestion of egg albumin by pepsin (2)

Salicylates have been found to inhibit the spreading effect of hyaluronidase in rheumatic fever patients (115, 116) Sodium salicylate inhibits the effect of hyaluronidase from bull testis and from *Cl. perfringens* *in vivo* and *in vitro*, but a higher concentration of salicylate is needed for the *in vitro* inhibition (77) Salicylates do not inhibit the effect of hyaluronidase on an elevated sedimentation rate produced in rabbits by the injection of sodium hyaluronate (283) This is evidence that salicylate does not exert a direct action of hyaluronidase It has been suggested that the inhibition of hyaluronidase *in vivo* may be due to the gentisate formed from salicylate (197), but there is conflicting evidence concerning the inhibition of the enzyme by gentisic acid (180, 234) Others confirm that neither salicylate nor gentisic acid inhibits hyaluronidase *in vitro* but that carboxyparabenzquinone does inhibit slightly at 0.001 molar and higher concentrations (180) This appears of no particular significance in therapy with salicylates since there is no evidence that carboxyparabenzquinone is formed in appreciable amounts.

*Effects of Micro-organisms* It has been known for some time that salicylates have antibacterial properties For example, salicylate was therefore suggested for the preservation of urine samples (22) Potassium salicylate, 0.2 molar, inactivates tomato spotted wilt virus and tobacco mosaic virus (25), and acetylsalicylic acid inactivates yellow fever virus (94), but the concentrations required are extremely high and the action is probably non-specific Sodium salicylate inhibits the growth of *Rickettsia typhi* in embryonated hen eggs and is comparable in effectiveness to para-aminobenzoic acid (230). Sodium salicylate markedly increases the oxygen consumption of tubercle bacilli (24), perhaps by serving as a substrate, acetylsalicylic acid is similarly effective, but only after hydrolysis Para-hydroxybenzoic acid has little and methyl salicylate and meta-hydroxybenzoic acid have no effect Sodium salicylate, 60 mgm per cent, inhibits the growth of the tubercle bacillus in a glycerin egg culture (199), the effect is additive with diaminodiphenylsulfone Whitehead, however, found that the growth of the tubercle bacilli *in vitro* is slightly enhanced by the addition of low concentrations of salicylate (273), and the observed inhibiting effect at higher concentrations may be nonspecific

The stimulating effect of salicylic acid on the tubercle bacillus is inhibited by p-aminosalicylic acid (184) Salicylic acid has some inhibitory effect on fungal

*Other Central Nervous System Effects* Several other symptoms presumably associated with stimulation or depression of the central nervous system have been described after the administration of salicylates. In cases of poisoning some patients are confused and incoherent (159, 252), and dizziness and irresponsibility have been noted. Tinnitus has long been found associated with large doses of salicylate. It begins in a range of 10 to 39 mgm per cent plasma concentration of salicylate (112). Diminished high-tone acuity may develop in a range of 10 to 46 mgm per cent, it occurred in all of 33 patients in the first 7 days at levels above 35 mgm per cent. Jager and Alway (148) observed tinnitus in 34 of 38 patients, but no serious toxic effects occurred below 40 mgm per cent. Prenatal medication with salicylates has been suggested as a factor in deafness of the newborn (259), but in four cases cited all the mothers had received quinine *ante partum* and none had received salicylates. Acetylsalicylic acid antagonizes the hypnotic action of phenobarbital in rats (105) whereas acetophenetidin does not, from studies on dogs it was concluded (120) that hypnotics should not be used in salicylate poisoning because the central nervous system is unusually susceptible to their action. Acetylsalicylic acid in doses comparable to therapeutic doses in man has little effect on spontaneous activity, maze learning or relearning of albino rats even when given throughout their life cycle (36). The thresholds of perception for touch, vibration, smell and hearing are not raised by therapeutic doses of acetylsalicylic acid (274). However, the two-point discrimination threshold was slightly raised in two subjects by 1.8 grams of acetylsalicylic acid. In doses of five to 40 mgm per kgm aspirin produces no change in the threshold for electrical convulsions in rabbits (256). The vasopressor effect of epinephrine is markedly increased by salicylates (194).

*Allergic Effects of Salicylate* Severe reactions from the administration of relatively small amounts of salicylates have repeatedly been reported. An asthmatic patient who took 0.3 gram of acetylsalicylic acid died within 10 minutes (83). She had had two previous severe reactions to the drug and knew that she was hypersensitive to it. The case was complicated by the fact that the patient had sarcoma of the dura. In another asthmatic patient death occurred after 0.6 gram of aspirin (97). This patient was also known to be hypersensitive to the drug. Hypersensitiveness to acetylsalicylic acid manifested by an angina pectoris syndrome has been described in two patients, one of them also had urticaria (244). A small "granule" of acetylsalicylic acid applied to the tongue produced a violent attack of coughing, asthma and itching within one minute in individuals hypersensitive to the drug (80).

The incidence of hypersensitivity to acetylsalicylic acid is probably no greater than two per 1000 of the general population (104). Hypersensitivity to acetylsalicylic acid is much less frequent than that to quinine (104), but the total number of cases is relatively large because of the large number of people who take acetylsalicylic acid. Buchstein and Prickman (48, 220) reviewed 62 allergic reactions to acetylsalicylic acid at the Mayo Clinic and 33 from the literature. Their survey did not include cases resulting from overdosage. They concluded that the skin test is dangerous and unreliable. The hypersensitivity may be remarkably

Rabbits sensitized to egg albumin are protected by acetylsalicylic acid from shock produced by the injection of a challenging dose of egg albumin (50) Nine of 10 sensitized animals given acetylsalicylic acid showed no shock, whereas all 10 controls responded with shock This observation has been confirmed, the administration of sodium gentisate does not prevent the shock but aminopyrine does (166) Salicylates have no effect on histamine shock (50)

If guinea pigs are injected with guinea pig brain and certain adjuvants, a progressive encephalomyelitis develops that cannot be prevented by salicylates, but it is usually prevented by a combination of salicylate and p-aminobenzoic acid (109)

*Acute Toxicity of Salicylates* As is true for many old and widely used drugs, less information on the acute toxicity in animals exists for salicylates than for more recently introduced drugs The fatal dose in guinea pigs (route of administration not stated) is approximately 1.5 gm per kgm (96) The  $LD_{50}$  for acetylsalicylic acid orally in mice is 1.36 gm per kgm (46) In white mice, the minimal lethal dose (route of administration not stated) of sodium salicylate is 5 millimoles per kgm (300 mgm per kgm), as compared with 2 millimoles per kgm for methyl salicylate and 0.5 millimoles per kgm for phenol (155) In rats, the oral  $LD_{50}$  for acetylsalicylic acid is 1.24 gm per kgm (47) The acute toxicity of sodium salicylate for rabbits is reduced by sodium bicarbonate and is possibly increased by magnesium trisilicate (69) Ascorbic acid, as the sodium or the calcium salt, reduces the toxicity of sodium salicylate (69, 114, 216) Magnesium salts reduced the toxicity of salicylates in mice (279), but a protective action of calcium gluconate in acetylsalicylic acid poisoning could not be demonstrated conclusively (19)

The single fatal dose of salicylates in man is not precisely known The fatal cases of poisoning have been reviewed in detail by Gross and Greenberg (113) The fatal dose of salicylate in man may be as low as 0.3 gram in individuals who are particularly hypersensitive to it, but patients have survived 40 grams of acetylsalicylic acid (173), and an arthritic patient is on record who took eight grams a day for 17 years without apparent harmful effects (70)

In his survey Lowy (181) found 1.6 deaths per million hospital admissions in the United States, attributed to aspirin This compares with 50 deaths per million admissions, attributed to barbiturates

*Therapeutic Effects* The inadequate demonstration that in normal human subjects acetylsalicylic acid raises the pain threshold for a circumscribed heat stimulus (134) provided tentative experimental confirmation for the long established use of salicylates for the relief of pain The major use of salicylates is for analgesia, particularly for treatment of simple headache or neuralgic pain

Whereas the salicylates are probably most frequently employed for the relief of headache and neuralgia, their use in the treatment of rheumatic fever is perhaps more important since few of the effective drugs are safe enough to be administered in sufficient dosage for the long periods of treatment required In considering the status of the salicylates in the therapy of rheumatic fever, it is interesting to recall that Latham (161) published a paper in *The Lancet* in 1885

infections when applied to the skin, and 36 of 54 patients were symptomatically cured (260). Of several salicylates tested some were effective in destroying the fungi associated with mildew diseases (60). In studies with *Streptococcus aureus* and *Escherichia coli*, evidence has been obtained that the antiseptic action is due to inhibition of the synthesis of pantothenic acid (144, 145). Pantothenic acid, pantolactone and pantoyltaurine antagonize the inhibiting effect of salicylate on *E. coli* (251) and pantothenic acid antagonizes the effect on *Mycobacterium tuberculosis* (146).

*Effects on Immunological Phenomena* Because of the favorable effect of salicylates in rheumatic fever, many investigators have been concerned with the effect of salicylates on immunological phenomena. A saturated solution of sodium salicylate neutralizes diphtheria and tetanus toxins without destroying their antitoxinogenic capacity (29). This may be due to protein denaturation, a known effect of high salicylate concentrations (6, 240). Toxic filtrates of hemolytic or non-hemolytic streptococci are not neutralized (72). Salicylates prevent precipitate formation when added to a system of egg albumin and its antibody (63). The inhibition is proportional to the concentration of salicylate. The effect is reversible and appears to be due to inactivation of the antibody.

Administration of two to three grams of acetylsalicylic acid per day has no appreciable effect on the development of agglutinins following typhoid inoculation (217). Guinea pigs and rabbits injected with rhesus monkey erythrocytes form less anti-Rh agglutinins when treated with sodium salicylate (141). Sodium salicylate given to pregnant mothers in doses of 8 to 10 grams a day for 20 weeks failed to prevent the development of erythroblastosis fetalis (195). Salicylates suppress antibody formation to typhoid H and O antigens in rheumatic fever patients injected with typhoid vaccine, the changes in leucocytes, plasma fibrinogen, erythrocytes, and sedimentation rate, which were observed in the controls, were absent (149).

Salicylates do not prevent experimental streptococcal arthritis in rabbits, but the animals manifest less joint inflammation (35). Sodium salicylate injected into rabbits suppresses the allergic dermal reaction to hemolytic streptococcal filtrates, but the subsequent proliferation of vascular lesions is the same as in the control animals (122).

In studies on immune body production in serum disease in rabbits, serum disease arthritis is usually prevented if salicylate treatment is started immediately after the serum injection and continued through the usual period of incubation (75). In similar experiments, others (263) found that salicylates do not prevent the histological changes. When sodium salicylate is injected into rabbits daily for eight days before administration of horse serum antigen, the formation of arterial lesions is prevented even though circulating antibody is present (254). It is postulated that salicylate prevents the fixation of antigen in the tissue cells. Some investigators found that sodium salicylate decreases the severity of the arterial and valvular lesions produced by the injection of horse serum into rabbits (249), others (231) found no prevention of either the vascular or the myocardial lesions but some prevention of the valvular lesions.

fall at plasma levels between 20 and 30 mgm per cent and a rapid fall at plasma levels between 30 and 40 mgm per cent. Usually two grams of sodium salicylate five times per day were given to maintain the high plasma levels. It was concluded that the drug effected a real cure in rheumatic fever. In similar studies on rheumatic fever patients, it was shown that large doses of salicylates do not reduce the relapse rate but that the patients are promptly relieved of pain and that the sedimentation rate usually returns to normal in a short time (269). The plasma salicylate levels were kept above 30 mgm per cent in these patients. Wégria and Smull (270, 271) treated rheumatic fever patients by keeping the plasma levels at 35 to 50 mgm per cent. They found that the course of the attack was ordinarily not shortened by the high doses of salicylates. Comparable results were obtained by Warren *et al* (268). Murphy (204) treated 12 patients with large doses of salicylates, and his results did not support the view that salicylates cause the subsidence of rheumatic joint inflammation. Keith and Ross (157) found that large doses of salicylate were effective in relieving the acute symptoms, but that they were ineffective in preventing cardiac lesions.

Salicylates have been shown (64) to be effective in preventing the development of the clinical manifestations of rheumatic fever in patients with intercurrent streptococcal pharyngitis. Only one of 47 such patients receiving five gm of sodium salicylate a day had a clinical attack of rheumatic fever whereas 57 of 139 control patients had clinical attacks.

Meyer and Ragan (196) studied the effects of the sodium salt of gentisic acid, a metabolic product of salicylate, in rheumatic fever patients. The compound had the same anti-rheumatic effect as the salicylates. Prothrombin time did not significantly decrease and the drug could not be detected in the blood. The doses used were of the order of 10 grams a day, which is probably far in excess of any amount originating from the salicylate doses ordinarily administered to rheumatic fever patients.

The spectacular results obtained with the adrenal cortical hormone, compound E, in rheumatic fever (135) and the possibility that adrenocorticotrophic hormone may have a favorable influence (135) raise the question of the future use of salicylates in this disease. It is too early to do more than speculate. Should these compounds prove to be effective consistently in rheumatic fever, then it remains to be seen whether they can be produced in adequate quantity and whether their long-continued administration will produce endocrine disturbances or other toxic effects. The inconvenience and pain of frequent parenteral administration will be factors in determining their use. While it is generally agreed that salicylates reduce the fever, relieve the pain, and arrest the inflammatory processes, there is insufficient evidence that they prevent cardiac complications, if compound E is effective in this respect, it will probably have a very important place in rheumatic fever therapy.

#### SUMMARY

The many intensive studies on the pharmacology of salicylates have revealed the principal features of their actions and physiological disposition. They are rapidly absorbed from all portions of the gastrointestinal tract, absorption being

entitled "Why does salicylic acid cure rheumatism?" and that 63 years later Reid published a paper (228) entitled "Does sodium salicylate cure rheumatic fever?" The salicylates are very effective in the symptomatic treatment of rheumatic fever (164), but it has been known for a long time (131) that other analgesics will also give prompt and effective relief of symptoms and may even provide permanent relief. Opinions are at variance on whether rheumatic fever as such responds to salicylates. Master and Romanoff (192) compared a group of rheumatic fever patients given 12 grams of sodium salicylate a day with a control group of patients receiving no salicylate and found no essential difference in the duration of the attacks. Acute pericarditis and myocarditis occurred with similar frequency in the two groups. In an attempt to assess the value of salicylate in rheumatic fever, it was found that in 86 per cent of 139 cases not receiving salicylate the symptoms ceased within four weeks, whereas 77 per cent of the 59 patients receiving salicylates not only had symptoms for the same length of time but suffered subsequent relapses (205). Relapses were rare, however, when the patients received more than eight grams a day. A group of 67 children receiving 13 grams of acetylsalicylic acid a day for six months gained more weight than did control patients. This was attributed to the increased comfort, and it was concluded that the salicylates were primarily of value in preventing pain (164). Roskam (235), who usually treated his patients with either eight grams of sodium salicylate or six grams of acetylsalicylic acid, found that acetylsalicylic acid was sometimes effective in patients resistant to sodium salicylate.

In a study of military personnel, a minimum concentration of 25 mgm per cent of salicylate had to be maintained in the plasma if the acute phase of the disease was to be suppressed (188). Ordinarily 10 grams of acetylsalicylic acid plus eight grams of sodium bicarbonate a day were given to maintain an average plasma level of 32 mgm per cent. The salicylates exert only a slight effect on the sedimentation rate in polycyclic attacks of rheumatic fever and such attacks do not respond as well as does the initial attack (49).

Coburn (61, 62) has suggested that rheumatic fever patients receiving large doses of salicylate for a long enough period of time are less prone to develop valvular heart lesions. He gave the drug intravenously to raise the plasma level rapidly to 40 mgm per cent. None of his 38 patients who received 10 grams of sodium salicylate daily and had plasma salicylate levels over 35 mgm per cent developed cardiac lesions, but on smaller doses of salicylate, 21 of 63 patients did develop valvular lesions.

Others (84) have commented on the studies of Coburn and criticized them (239), claiming that his criteria of cardiac damage were not adequately described, that the follow-up period was too short, that the criteria for classifying cases as severe or mild were not precisely stated, and that in some patients the sedimentation rate did not return to normal.

Doses of 0.1 gram of salicylate per pound body weight, which produce plasma levels of 30 to 45 mgm per cent, are effective in rheumatic polyarthritis and acute rheumatic carditis in children (257), but smaller doses are ineffective. Using the erythrocyte sedimentation rate as the criterion of effectiveness, Reid (228) found no change when plasma salicylate levels were less than 20 mgm per cent, a slow

12. BARBOUR H G Antipyretics III Acetylsalicylic acid and heat regulation in fever cases. Arch. Internal Med. 24 624-32 1919
14. BARBOUR, H G The heat regulating mechanism of the body. Physiol. Rev. 1 295-326 1921
15. BARBOUR H G Heat regulation and water exchange. XI. Blood and plasma changes in cocaine and *B. coli* fevers and aspirin antipyresis. J. Pharmacol., 29 427-40, 1926.
16. BARBOUR, H G AND DEWENT, M. M Antipyretics. II Acetylsalicylic acid and heat regulation in normal individuals. Arch. Internal Med. 24 617-23 1919
17. BARBOUR H G AND DICKERSON, V Gastric ulceration produced in rats by oral and subcutaneous aspirin. Arch. int. pharmacodyn., 58 78-87 1933.
18. BARBOUR, H G AND FISK, M. E. Liver damage in dogs and rats after repeated oral administration of cinchophen ethyl ester of paramethylphenyl-cinchoninic acid (tolylan) and sodium salicylate. J. Pharmacol., 48 341-57 1933.
19. BARBOUR, H G AND PORTER J A An investigation of the possible protective action of calcium gluconate in aspirin poisoning. J. Pharmacol. 60 224-7 1937
20. BARNETT H L, POWERS J R, BENWARD, J H AND HARTMAN, A. F Salicylate intoxication in infants and children. J. Pediat., 21 214-23 1942.
21. BAXTER, E. H, HARTWELL R. M AND RECK L. E. Methylsalicylate poisoning. J. Am. Med. Assoc. 111:2476-7, 1933
22. BEHRE, J. A. AND MUEHLBERG, W Urinary preservatives including hexamethylenamine. J. Lab. Clin. Med., 11: 785-90 1926.
23. BELISLE, M. Fièvre rhumatismale acid para aminobenzoïque. Union Med. Canada, 77: 392-425, 1943.
24. BERNHEIM, F The effect of various substances on the oxygen uptake of the tubercle bacillus. J. Bact. 41 387-95, 1941.
25. BEST R. J Inactivation of tomato spotted wilt virus by salicylate. Australian J. Exper. Biol. Med. Sci., 24. 27-31 1946.
26. BEUTNER, R., CALLEATE, B, POWELL, E AND BORTIN, L. On the absorption and excretion of methyl salicylate administered by inunction. J. Lab. Clin. Med., 23 1655-63 1943
27. BEYER K. H., MATTIS, P. A., PATCH, E. A. AND RUSCO, H. F Para aminohippuric acid its pharmacodynamic actions. J. Pharmacol., 84. 136-46, 1945
28. BIDDLE, E Aspirin poisoning. Brit. Med. J., 1365, 1933.
29. BIRKHAU E D Detoxifying and disinfecting properties of sodium salicylate. Its action on diphtheria and tetanus toxins and on streptococcal "toxic" filtrates. J. Infectious Diseases, 48 212-25, 1931
30. BLUME, W AND BRUNZIO P. Vergleichende Untersuchungen über den Resorptionsverlauf der Salicylsäure nach intratrachealer und nach subkutaner Injektion. Arch. exper. Path. Pharmacol., 161 191-95, 1931.
31. BLUME, W AND BRUCHOLD G Über den Resorptionsverlauf der Salicylsäure in der Mundhöhle. Arch. exper. Path. Pharmacol. 166 472-92, 1932.
32. BLUME, W AND FISCHER F W Über den Resorptionsverlauf der Salicylsäure nach intramuskulärer Injektion und über die Resorptionsfähigkeit der Harnblase für Salicylsäure. Arch. exper. Path. Pharmacol. 179: 646-54, 1935
33. BLUME W AND NOHARA, F S. Vergleichende Untersuchungen über den Resorptionsverlauf der Salicylsäure nach peroraler und rektaler Applikation. Arch. exper. Path. Pharmacol. 173 413-30, 1933
34. BLUME, W AND PLUM, K Vergleichende Untersuchungen über den Resorptionsverlauf der Salicylsäure nach intrapleuraler und intraperitonealer Applikation. Arch. exper. Path. Pharmacol. 179 655-61, 1935.
35. BOOTS, R. AND SWIFT, H. The effect of sodium salicylate on the development of arthritis in rabbits inoculated with *Streptococcus viridans*. J. Am. Med. Assoc., 78 1922, 1922.
36. BOUGHTON L. L. The effects of life cycle therapeutic dosage administration of drugs to albino rats. II. On activity, maze learning and relearning. J. Am. Pharmaceut. Assoc. 31 240-4, 1942.
37. BOWEN B D, ROUTA, J F AND CLINCK, O W The differential diagnosis of salicylate poisoning and diabetic acidosis. J. Am. Med. Assoc., 107 276-7, 1936.
38. BOWMAN, K. M. A case of psychosis due to large doses of acetyl salicylic acid. J. Nervous Mental Diseases, 78: 57-60, 1923
39. BOTLE M V, SMULL, K. AND WIGGIA R The effect of sodium salicylate on the acid base balance of the blood. Am. J. Med. 3 31-3 1947
40. BRADLEY W B A method for the rapid determination of salicylates. Proc. Soc. Exper. Biol. Med., 35 1-4 1935
41. BRADLEY, W B, SCHNEIDORF J G AND IVY A C III The rate of absorption of salicylates and the effect of certain compounds on the rate of absorption of acetylsalicylic acid from the stomach and intestine. Am. J. Digestive Diseases, 3 415-9, 1936
42. BRAT, H G, RYMAN B E AND THORPE W V The fate of certain organic acids and amides in the rabbit 5-o- and m-hydroxybenzoic acid and amides. Biochem. J., 43. 561-7 1945
43. BRADIE, B B, LUDWIG S. AND COOPER A. F The determination of salicylic acid in plasma. J. Pharmacol. 80 114-7 1944
44. BROWN E W AND SCOTT W O The absorption of methyl salicylate by the human skin. J. Pharmacol. 50: 32-40 1934.
45. BROWN E W AND SCOTT, W O The comparative absorption of certain salicylate esters by the human skin. J. Pharmacol. 50 373-55 1934.
46. BROWNLEE G A comparison of the antipyretic activity and toxicity of phenacetin and aspirin. Quart. J. Pharm. Pharmacol. 10: 609-20 1937

most rapid in the small intestine from slightly acid solutions. Distribution is throughout the body, appreciable amounts being within the cells. Degradation includes partial oxidation or conjugation with glycine or glycuronic acid. Excretion is moderately rapid, the free salicylate being excreted more rapidly when the urinary pH is high.

The salicylates are among the least toxic of commonly used drugs, but rarely, even in moderate doses, they produce severe reactions, particularly in asthmatic patients. Large doses may produce a transient fall in plasma prothrombin, but there is seldom a hemorrhagic tendency. Nausea and vomiting are usually associated with high plasma levels and are primarily central in origin. Large doses of salicylates stimulate the respiration, this produces an alkalosis which may continue until a ketosis develops.

Several decades of research on the salicylates have left many problems still unsolved. One of the most important is whether the salicylates act *per se* or through the degradation product, gentisic acid. In favor of the latter hypothesis is the beneficial action of gentisic acid in rheumatic fever (196). But there is some question whether the compound inhibits hyaluronidase (180, 197, 234), and the dose required in therapy is larger than would be expected on the basis of the fraction of salicylate known to be converted to gentisate (154), also the drug fails to prevent anaphylactic shock (166) whereas the salicylates are effective (50, 166).

The metabolism of methyl salicylate has not been studied as intensively as that of salicylic acid and acetylsalicylic acid. It may be that it differs appreciably.

The failure to demonstrate consistently an analgesic effect of salicylates may be due to inadequate methods of studying analgesia.

The demonstration that salicylates prevent anaphylactic shock raises the question of the possible relation of this phenomenon to the mechanism of salicylate action in rheumatic fever.

#### REFERENCES

1. AGLAND, T. D. Delirium following the treatment of acute rheumatism by salicylic acid. *Brit. Med. J.*, 1: 337, 1931.
2. ALPERT, S. AND MARTIN, G. J. A comparative study of the inhibitory action of chemical agents on peptic activity. *Am. J. Digestive Diseases*, 16: 10-14, 1949.
3. ANDERSEN, A. H., ANDERSEN, E. AND BURN, G. C. Mechanism of the toxic action of salicylic acid. *Acta Med. Scand.*, 109: 336-64, 1941.
4. ANDREWS, H. L. AND WORKMAN, W. Pain threshold measurements in the dog. *J. Pharmacol.*, 73: 99-103, 1941.
5. ANGELICO, R. Sulle trasformazioni dell'acido salicilico nell'organismo animale. *Arch. farmacol. sper.*, 31: 8-12, 1921.
6. ANSON, M. L. AND MIRSKY, A. D. The equilibria between native and denatured hemoglobin in salicylate solutions and the theoretical consequences of the equilibrium between native and denatured protein. *J. Gen. Physiol.*, 17: 399-408, 1934.
7. ASHWORTH, C. T. AND McKEMIE, J. F. Hemorrhagic complications with death probably from salicylate therapy. Report of two cases. *J. Am. Med. Assoc.*, 126: 803-10, 1944.
8. BAKER, Z. AND KING, C. G. The purification, specificity and inhibition of liver esterase. *J. Am. Chem. Soc.* 57: 358-61, 1935.
9. BALDONT, A. Sulle trasformazioni dell'acido salicilico nell'organismo animale. *Arch. farmacol. sper.*, 17: 241-7, 1914.
10. BALDONT, A. Sull'eliminazione dell'acido salicilurico e dell'acido salicilico in seguito a somministrazione di acido salicilico salicilato di sodio e di placebo. *Arch. farmacol. sper.*, 18: 151-77, 1914.
11. BALDONT, A. Sul comportamento dell'acido salicilurico nell'organismo umano. *Biochim. therap. sper.* 10: 271-5, 1923.
12. BALDONT, A. Sulla sintesi dell'acido salicilurico in alcuni stati morbose. *Biochim. therap. sper.* 10: 335-49, 1923.



56. ERMAS A Methyl salicylate poisoning in an infant Report of a patient with partial necropsy *J. Pediat.* 13 550-4, 1938
57. ERCOLI V AND LEWIS M V Studies on analgesics. I The time-action curves of morphine, codeine, dihydriodide and demerol by various methods of administration II Analgesic activity of acetylsalicylic acid and aminopyrine. *J. Pharmacol.* 84 301-17, 1945
58. ERGANDIAN J A FORBES G B AND CASE, D M Salicylate intoxication in the infant and young child. A report of thirteen cases. *J. Pediat.*, 30 129-45 1947
59. ERSTENE, A C Erythrocyte sedimentation rate plasma fibrinogen and leucocytosis as indices of rheumatic infection. *Am. J. Med. Sci.*, 180 12-24 1930.
60. FALCONER, J S. AND TAYLOR D B The initial stages in the purification of pig liver esterase. *Biochem. J.*, 40- 831-4 1946.
61. FARRER, H R., YIENGST, M J AND SHOCK N W The effect of therapeutic doses of aspirin on the acid base balance of the blood in normal adults. *Am. J. Med. Sci.*, 217 256-62 1949
62. FASHEA G J AND WALKER, J N Salicylate intoxication. Studies on the effects of sodium salicylate on prothrombin time and alkali reserve. *Am. J. Diseases Children* 68 369-75 1944
63. FIELD J B Hypoprothrombinemia induced in suckling rats by feeding 3' methylenebis (4-hydroxyooumarin) and acetylsalicylic acid to their mothers *Am. J. Physiol.* 143 238-42 1945
64. FINDLAY G M The action of certain surface-acting substances on yellow fever virus (neurotropic strain) preliminary observations *Trans. Roy. Soc. trop. Med. Hyg.* 36 247-52 1943
65. FINE, M S. AND CHACE A F The influence of salicylates upon the uric acid concentration of the blood *J. Biol. Chem.*, 21 371-5 1915
66. FLOEKHOFF F C An experimental study of aspirin, a new salicylic acid preparation. *Med. News N. Y.*, 75 645-7 1929
67. FRANCIS, V GHENT O T AND BULLEN, S. S. Death from ten grains of aspirin. *J. Allergy* 6 504-6 1935.
68. FRIEDENBERG C Die quantitative Bestimmung der Salicylsäure im Blute und deren Wirkungen auf das Herz *Arch. exper. Path. Pharmacol.*, 80 235-53 1917
69. FRIEDMAN M Observations concerning the effect of (1) sodium salicylate and (2) sodium salicylate and glycine upon the production and excretion of uric acid and allantoin in the rat. *Am. J. Physiol.*, 152 302-8 1948
70. FRIEDMAN M AND BYERS S. O Effect of sodium salicylate upon the uric acid clearance of the Dalmatian dog *Am. J. Physiol.*, 154 187-9 1943
71. GALIMARD J E. Métabolisme du salicylate de sodium salicylure et glucuronie. *Bull. soc. chim. biol.* 26 185-92, 1944.
72. GALIMARD J E Métabolisme du salicylate de sodium. III. Adsorption *in vitro* per le sérum sanguin *Bull. soc. chim. biol.* 27 206-11 1945
73. GALIMARD, J E Metabolisme du salicylate de sodium V Effet du salicylate sur la production et l'excrétion de l'acide urique. *Bull. soc. chim. biol.* 28: 153-60, 1946
74. GARDNER, E AND BLANSTON W B The incidence of aspirin hypersensitivity *Am. J. Med. Sci.* 200- 390-4 1940.
75. GILMAN, A. AND BARBOUR H G Effects of phenacetin and aspirin respectively upon action of phenobarbital. *Proc. Soc. Exper. Biol. Med.* 32. 1634-6 1935.
76. GOETTL, F R The experimental evidence for analgesic properties of antipyretic drugs. A critical review of literature with report on additional experiments. *Permanent Foundation Med. Bull.* 4 49-53 1946.
77. GOETTL, F R., BURRILL D Y AND IVY A C A critical analysis of algometric methods with suggestions for a useful procedure. *Northwestern Univ. Bull.* 17 250-9 1943
78. GOETTL F R AND PARSONS C M The influence of acetylsalicylic acid upon analgesic effects of induced pain. *Proc. Am. Fed. Clin. Res.*, 2 92-3 1945.
79. GOON R A CAMPBELL B AND GOON T A Prophylaxis of allergic acute disseminated encephalomyelitis in guinea pigs with salicylate and para aminobenzoic acid *Fed. Proc.*, 8 356 1949
80. GOVAN C D The effect of salicylate administration on the prothrombin time. *J. Pediat.*, 29 629-36 1946.
81. GRABFIELD, G P A pharmacologic study of the mechanism of gout. *Ann. Internal Med.* 11 651-6 1937
82. GRAHAM J D P AND PARKER W A. The toxic manifestation of sodium salicylate therapy *Quart. J. Med.*, 17 153-63 1948
83. GROSS M AND GREENBERG, L A The salicylates A critical bibliographic review, xvii + 380 pp., Hillhouse Press New Haven 1949
84. GUNNER, R. AND SIDDS, M Therapeutic measures in rheumatic fever A comparative study of one hundred and fifty cases. *New Engl. J. Med.*, 233 652-7 1945
85. GUERRA, F The action of sodium salicylate and sulfadiazine on hyaluronidase. *J. Pharmacol.* 87 193-7, 1946
86. GUERRA F Hyaluronidase inhibition by sodium salicylate in rheumatic fever *Science* 103 686-7, 1946
87. GUERRA F AND BARBOUR, H. G The mechanism of aspirin antipyretic in monkeys. *J. Pharmacol.*, 79- 55-61, 1943
88. GUERRA, F AND BROBECK J R. The hypothalamic control of aspirin antipyretic in the monkey *J. Pharmacol.* 30- 200-16 1944
89. GUEST G M RAPOPORT, S AND ROSCOE C Clinical and experimental studies of salicylate poisoning *Am. J. Diseases Children* 64. 200-1 1942
90. GUEST G M. RAPOPORT S. AND ROSCOE C. The effect of salicylates on the electrolyte structure of the blood plasma. II. The action of therapeutic doses of sodium salicylate and of acetylsalicylic acid in man. *J. Clin. Invest.*, 24. 770-4, 1945.

- 47 BROWNLEE, G AND GADSDEN, J H A therapeutic index for the coal tar antipyretics with an appendix on the accuracy of a cross-over test. *Quart. J Pharm Pharmacol*, 12 45-70, 1939
- 48 BUCHSTEIN, H F AND PRICKMAN, L E Allergic reactions from acetylsalicylic acid (aspirin). *Proc. Staff Meetings Mayo Clin*, 12 618-8, 1937
- 49 BUTT, H R, LEAKE W H, SOLLEY, R F, GRIFFITH, G C, HUNTINGTON, R. W AND MONTGOMERY, H. Studies in rheumatic fever I The physiologic effect of sodium salicylate on the human being, with particular reference to prothrombin level of blood and the effect of hepatic parenchyma. *J Am Med Assoc*, 128 1195-1200, 1945
- 50 CAMPBELL, B Inhibition of anaphylactic shock by acetylsalicylic acid. *Science*, 168 478-9, 1948.
- 51 CARAYATI, C M Gastric endoscopic and secretory findings during salicylism. *Gastroenterology*, 6 7-8, 1946.
- 52 CARAYATI, C M AND COSONOVE, E F Salicylate toxicity The probable mechanism of its action. *Ann. Internal Med*, 24 638-42, 1946
- 53 CARAYATI, C M AND WHIME, C B General manifestations of salicylism. *Southern Med. J*, 38 722-6, 1945.
- 54 CHABANIER, H, LEBERT, M AND LOBO-ONELL, C De l'adsorption du salicylate de sodium par le sérum sanguin. *Compt rend soc biol* 88 178-80, 1923
- 55 CHABANIER, H, LEBERT, M AND LOBO-ONELL, C De l'adsorption de l'ion salicylique par le sérum sanguin in vivo Du mode de sécrétion de l'ion salicylique par le rein. *Compt. rend soc. biol.*, 88: 603-10, 1923
- 56 CHARTERS, A D A case of aspirin poisoning. *Brit. Med J*, 10-1, 1944
- 57 CLARK, B B, ADAMS, W L AND ROMANO, J J The effect of acetylsalicylic acid on gastric secretion. *Gastroenterology*, 9 461-5, 1947
- 58 CLARK, B B AND SPITALNY, M Prothrombinopenic activity of the salicylates and pharmacologically related drugs. *Fed Proc.*, 5 171, 1946
- 59 CLAUGEN, F W AND JACOB, B V The relation of the plasma salicylate level to the degree of hypoprothrombinemia. *J Lab Clin. Med.*, 31 428-38, 1945
- 60 CLAYTON, E E Fungicidal value of the salicylates. *Science*, 96 366, 1942
- 61 COBURN, A F Salicylate therapy in rheumatic fever A rational technique. *Bull Johns Hopkins Hosp.*, 73 435-64, 1943.
- 62 COBURN, A F The rheumatic fever problem I Present status. *Am J Diseases Children*, 70 339-47, 1943.
- 63 COBURN, A F AND KAPP, E M The effect of salicylates on the precipitation of antigen with antibody. *J Exper Med*, 77: 173-83, 1943
- 64 COBURN, A F AND MOORE, L V Salicylate prophylaxis in rheumatic fever. *J Pediat.*, 21 180-3, 1942
- 65 COOAN, D G AND HIRSCH, E O The cornea. VII Permeability to weak electrolytes. *Arch. Ophthalm.*, 32: 276-82, 1944
- 66 COHEN, P P AND MCGILVER, R W Peptide bond synthesis III On the mechanism of p-aminhippuric acid synthesis. *J Biol Chem*, 171: 121-33, 1947
- 67 COOKE, F S, HIGLEY, C S AND WARREN, H A The magnitude of salicylate hypoprothrombinemia. *Proc. Am Fed. Clin Res.*, 2 57-8, 1945
- 68 COOKE, F S, WARREN, H A AND HIGLEY, C S Toxicity of salicylates. *J Lab Clin Med*, 30 378-9, 1945.
- 69 CRONHEIM, G, WILAND, J AND EHRLICH, M The metabolism and toxicity of salicylic acid in combination with various drugs. *J Am Pharm Assoc., Sci Ed* 33 451-5, 1949
- 70 CUNNINGHAM, E M Acetylsalicylic acid deaths. *J Am Med Assoc.*, 116 776, 1941
- 71 DANIELS, A L AND EVERSON, G J Influence of acetylsalicylic acid (aspirin) on urinary excretion of ascorbic acid. *Proc Soc. Exper Biol. Med.*, 35 20-4, 1936
- 72 DAVIS, D J The effect of sodium salicylate on various types of experimental arthritis. *Arch. Internal Med.*, 15: 555-7, 1915.
- 73 DENIS, W The influence of salicylates on the elimination of uric acid and other waste products from the blood. *J Pharmacol.*, 7 255-62, 1915
- 74 DENIS, W AND MEANS, J H The influence of salicylate on metabolism in man. *J Pharmacol*, 8 272-83, 1916.
- 75 DERICK, C L, HITCHCOCK, C H AND SWIFT, H F The effect of antirheumatic drugs on the arthritis and immune body production in serum disease. *J Clin Invest.*, 5: 437-40, 1925
- 76 DODD, K., MINOT, A S AND ARENA, J M Salicylate poisoning, an explanation of the more serious manifestations. *Am. J Diseases Children*, 53 1435-46, 1937
- 77 DORFMAN, A., REIMERS, E J AND OTT, M L Action of sodium salicylate on hyaluronidase. *Proc. Soc. Exper Biol Med*, 64 357-60 1947
- 78 DRY, T J, BUTT, H R AND SCHEFFLEY, C H The effect of oral administration of para-aminobenzoic acid on the concentration of salicylates in the blood. *Proc. Staff Meetings Mayo Clinic*, 21 497-504, 1946.
- 79 DRYE, R., PAUL, W D AND ROUTH, J I Effect of acetylsalicylic acid ingestion on electrophoretic patterns of human plasma. *Proc Soc Exper Biol Med.*, 66 532-4, 1947
- 80 DUKE, W W Aspirin allergy A method of testing for aspirin sensitivity and a method of avoiding aspirin catastrophes. *J Allergy* 4 428-7, 1932.
- 81 DWYER, H L Oil of wintergreen (methyl salicylate) poisoning. *J Kansas Med Soc.*, 34: 168-7, 1933
- 82 DYKE, B. C A case of acetylsalicylic acid (aspirin) poisoning. *Lancet*, 279: 618-4, 1935.
- 83 DYAR, B. R. Death following ingestion of five grains of acetylsalicylic acid. *J Am. Med Assoc.*, 101: 446, 1933
- 84 Editorial Salicylate therapy in rheumatic fever. *J Am Med Assoc.*, 138: 357, 1948
- 85 EGGLSTON, C AND HATCHER, R. A The set of the emetic action of various drugs. *J Pharmacol.*, 7 225-53, 1912

- 153 KELLER, J W, JR. A rapid method for the determination of salicylates in serum or plasma. *Am. J. Clin. Path.*, 17 415-7, 1947.
- 159 KRASSOFF, S O AND BERNSTEIN M. Acetylsalicylic acid poisoning with a report of a fatal case. *J. Am. Med. Assoc.*, 135 712-4, 1947
- 160 KUTZER, L AND RICHARDS, R. K. A new method for the evaluation of drugs affecting the reaction of mice to pain stimulation. *J. Lab. Clin. Med.*, 23 1585-90 1943
- 161 LATHAM, P W Why does salicylic acid cure rheumatism? *Lancet* 1 1153-50, 1935
- 162 LAWSON, R. B AND KAISER, A. D Methyl salicylate poisoning. Report of a case of death in a child with rheumatic fever following the cutaneous administration of oil of wintergreen. *Arch. Pediat.*, 54 509-13, 1937
- 163 LEAS, R D Reducing substances in the urine. *J. Lab. Clin. Med.*, 12 15-17 1926
- 164 LEECH, C B The value of salicylates in prevention of rheumatic manifestation. *J. Am. Med. Assoc.* 95 932-4, 1930.
- 165 LERMAN, A. J Respiratory, inhibitory and paralytic effects of some barbitol hypnotics and coal tar analgesics. *J. Pharmacol.*, 65 235-42 1939
- 166 LEIFFER, R M AND MILLER, B F Personal communication
- 167 LEITER, D The non appearance of salicylates in the urine after the administration of dicumarol. *J. Biol. Chem.*, 154 305-6, 1944.
- 168 LEITER, D, LOLL, G AND GREENBERG, L. A. The fate of acetylsalicylic acid. *J. Pharmacol.*, 87: 329-42, 1946.
- 169 LEULIER, A., SOHIER, R. AND NOUVEL, G Salive parotidienne et salicylate de sodium. *Compt. rend. soc. biol.*, 140: 874-5 1946.
- 170 LIGHT, J. A. AND HOOKER, S. P Effect of acetyl salicylic acid on sedimentation rate of erythrocytes in rheumatic fever. *Proc. Soc. Exper. Biol. Med.*, 48: 69-70, 1941
- 171 LIGON, E. W, JR., MADDEN, R. J DAVIS, P L. AND SMITH, P K. Absorption of salicylates from intestinal loops in the dog. *Fed. Proc.* 7 240, 1943.
- 172 LINK, K. P, OVERMAN, R. S, SULLIVAN, W R., HUBNER, C F AND SCHEEL, L. D Studies on the hemorrhagic sweet clover disease. XI. Hypoprothrombinemia in the rat induced by salicylic acid. *J. Biol. Chem.*, 147 463-74, 1943
- 173 LIPKIN, S. Survival after 600 grains of aspirin. *Lancet*, 226 923 1934.
- 174 LIVON, C. Recherches sur l'action physiologique de l'acide salicylique sur la respiration. *Compt. rend.*, 90 321, 1880.
- 175 LOBERG, E. Eine klinische Methode zur quantitativen Bestimmung von Salicylsäure in Blutserum und Liquor cerebrospinalis. *Biochem. Z.*, 170 173-84, 1926.
- 176 LODENKAMPER, H. Pharmakologische und klinische Untersuchungen zur Salicylsäuretherapie. *Z. ges. exper. Med.*, 98 247-54, 1936.
- 177 LOLL, G AND SMITH, R. Effervescent mixtures as adjuvants to the rapid absorption of ingested drugs. *New Engl. J. Med.*, 235: 80-4, 1946.
- 178 LONGENCKER, H. E, FRICK, H. H. AND KING, C G The effect of organic compounds upon vitamin C synthesis in the rat. *J. Biol. Chem.*, 135: 497-510, 1940.
- 179 LONGENCKER, H. E, MCGULIN, R. R., TOLLY, R. H., 3RD, AND KING, C G An acceleration of vitamin C synthesis and excretion by feeding known organic compounds to rats. *J. Biol. Chem.*, 129: 445-53, 1939
- 180 LOWENTHAL, J AND GAGNON, A. The inhibition of hyaluronidase by sodium salicylate and its possible metabolites. *Can. J. Res. E.*, 26 200-5, 1943.
- 181 LOWY, O A comparative study of the habitual use of barbiturates and coal tar derivatives as furnished by reports from various hospitals throughout the United States. *Can. Med. Assoc. J.*, 31 638-41, 1934.
- 182 LUTWAK MANN, C The effect of salicylate and cinchophen on enzymes and metabolic processes. *Biochem. J.*, 36 706-28, 1942.
- 183 LUTWAK MANN, C. The excretion of a metabolic product of salicylic acid. *Biochem. J.*, 37: 246-8, 1943.
- 184 LUTZ, A Recherches sur le mode d'action de l'acide salicylique et de l'acide p-aminosalicylique dans l'inhibition de la croissance du bacille tuberculeux. *Ann. Inst. Pasteur*, 76: 150-67 1949
- 185 MACKAY, E. M., WICK, A. N., CAERNY, H. O AND BAERNUM, C P The influence of alkalosis and acidosis upon fasting ketosis. *J. Biol. Chem.*, 138. 63-8, 1941.
- 186 MADISON, H. Über pathologische histologische Befund bei therapeutischer und experimenteller Natriumsalicylatvergiftung sowie deren günstige Beeinflussung durch Traubenzucker. *Deut. Arch. klin. Med.*, 176 612-25, 1934.
- 187 MAGGIORI, G F Some observations on salicylate therapy in children. *Biochem. J.*, 41 lii, 1947
- 188 MANCHESTER, R. C Rheumatic fever in naval enlisted personnel. II. Effectiveness of intensive salicylate therapy in cases of acute infection. *Arch. Internal Med.*, 73 170-80, 1946
- 189 MANCHESTER, R. C Rheumatic fever in naval enlisted personnel. III. The physiologic and toxic effects of intensive salicylate therapy in acute cases. *J. Am. Med. Assoc.*, 131 209-13, 1945
- 190 MANDEL, A. R. Xanthin as a cause of fever and its neutralization by salicylates. *Am. J. Physiol.*, 20 432-43, 1907
- 191 MARTIN, G J AND BYERS, S. Biochemical studies of salicylic acid and a series of its derivatives. *Am. J. Digestive Diseases*, 15 127-31 1943
- 192 MASTER, A. M AND ROMANOFF A. Treatment of rheumatic fever patients with and without salicylates. *J. Am. Med. Assoc.*, 98 1978-80, 1932.
- 193 MAUGHAN, G B, EVELYN, K. A. AND BROWNE, J. S. L. A method for the quantitative estimation of glucuronic acid and conjugated glucuronides. *J. Biol. Chem.* 126 567-72 1933

- 121 HAAG, H B AND LARSON, P S Studies on the fate of nicotine in the body I The effect of pH on the urinary excretion of nicotine by tobacco smokers J Pharmacol, 76 235-9, 1942
- 122 HAGEBUH, O E AND KINSZLA, R A Effect of sodium salicylate on intradermal reactions of rabbits. Proc. Soc. Exper Biol Med., 27: 922-4, 1930
- 123 HALPERN, B N, GAUDIN, O AND STIFFEL, C Étude de l'influence des cations sur la perméabilité cutanée et cellulaire vis-à-vis de certains dérivés salicylés. Recherches sur l'absorption cutanée chez l'Homme et l'animal. Compt. rend. soc. biol., 142 819-21, 1948
- 124 HALSTROM, F AND MÖLLER, K. O Ein Fall von tödlicher Salicylsäurevergiftung (Acetylsalicylsäure?) mit quantitativer Salicylsäurebestimmung in den Organen. Dcut. Z. ges. gerichtl. Med., 31 182-8, 1939
- 125 HANZLIK, P J The salicylates IX The question of salicyluric acid in salicyl urines. J Pharmacol., 10 461-90, 1917
- 126 HANZLIK, P J The action and uses of salicylates and cinchophen in medicine. Medicine, 5 197-373, 1926.
- 127 HANZLIK, P J Medicine monographs, vol 9, Actions and uses of the salicylates and cinchophen in medicine, xiii + 200 pp., Williams and Wilkins, Baltimore, 1927
- 128 HANZLIK, P J, DE EDS, F AND PRESHO, E. Urinary excretion of salicyl after the administration of salicylate and salicyl esters. Proc. Soc. Exper Biol. Med., 19: 303-4, 1922.
- 129 HANZLIK, P J AND PRESHO, N E The salicylates. XIV Liberation of salicyl from and excretion of acetyl-salicylic acid J Pharmacol., 21: 247-61, 1923
- 130 HANZLIK, P J AND PRESHO, N E The salicylates. XVI. Liberation of salicyl from and excretion of methyl salicylate with a note on the irregular toxicity of the ester in man J Pharmacol., 26 71-81, 1925
- 131 HANZLIK, P J, SCOTT, R. W AND GAUCHAT, P C The salicylates X The specificity of salicylate in rheumatic fever J Lab Clin. Med., 4 112-22, 1918
- 132 HANZLIK, P J AND WETZEL, N C The salicylates. XI The stability and destruction of the salicyl group under biological conditions. J Pharmacol., 14. 25-42, 1920
- 133 HANZLIK, P J AND WETZEL, N C The salicylates XII The excretion of salicyl after the administration of methyl salicylate to animals J Pharmacol., 14. 43-6, 1920
- 134 HARDY, J D WOLFF, H G AND GOODELL, H Studies on pain A new method for measuring pain threshold Observations on spatial summation of pain J Clin. Invest., 19 649-57, 1940
- 135 HENCH, P S, SLOCUM, C H, BARNES, A R., SMITH, H L, POLLEY, H F AND KENDALL, E C The effects of the adrenal cortical hormone 17-hydroxy 11-dehydrocorticosterone (compound E) on the acute phase of rheumatic fever Preliminary report. Proc. Staff Meetings. Mayo Clinic, 24 277-97, 1949
- 136 HETN, L G Intrarectal administration of sodium salicylate in acute rheumatic fever with satisfactory results. J Am Med Assoc., 63 1004-5, 1914
- 137 HOFFMAN, W S, POMERANC, M., VOLINI, I F AND NOBE, C Treatment of acute rheumatic fever with aspirin with special reference to the biochemical changes. Am J Med., 6: 433-42, 1949
- 138 HOLMES, E G A contribution to the study of the metabolism of salicylic acid J Pharmacol., 26: 297-314, 1925.
- 139 HOMBURGER, F Effect of sodium salicylate on sedimentation rate of erythrocytes in vitro. Am J Med. Sci., 210 163-72, 1945
- 140 HOMBURGER, F The effect of sodium salicylate on the sedimentation rate of erythrocytes in vivo Am J Med. Sci., 211 346-49, 1946
- 141 HOMBURGER, F Sodium salicylate inhibiting anti Rh immunization in animals. Proc. Soc. Exper Biol. Med., 61 101-2, 1946
- 142 HUGHES, R C A case of methyl salicylate poisoning Can Med Assoc. J., 27 417-8, 1932.
- 143 HUNTINGTON, R. W, RYAN, R. D, BUTT, H R., GRUFFITH, G C, MONTGOMERY, H, SOLLEY, R. F AND LEAKE, W H: Studies in rheumatic fever II Absorption of salicylates. Ann Internal Med., 24: 1029-38, 1946
- 144 IVÁNOVICS, G Das Salicylat-Ion als spezifischer Hemmungsstoff der Biosynthese der Pantothenäure. Z. physiol Chem., 276 33-35, 1942.
- 145 IVÁNOVICS, G Mechanismus der antiseptischen Wirkung der Salicylsäure Naturwissenschaften, 30 194, 1943.
- 146 IVÁNOVICS, G Antagonism between effects of p-aminosalicylic acid and salicylic acid on growth of M. tuber culosis. Proc. Soc. Exper Biol. Med., 79 482-3, 1949
- 147 JACKSON, A V Toxic effects of salicylate on the foetus and mother J Path. Bact., 60: 537-63 1948
- 148 JACOB, B V AND ALWAY, R The treatment of acute rheumatic fever with large doses of sodium salicylate with special reference to dose management and toxic manifestations. Am J Med Sci., 211: 973-85, 1946
- 149 JACOB, B V AND NICKERSON, M The altered response of human beings to the intramuscular administration of typhoid vaccine during massive salicylate therapy Am J Med., 3: 408-22, 1947
- 150 JAILER, J W, ROSENFELD, M AND SHANNON, J A. The influence of orally administered alkali and acid on the renal excretion of quinine, chloroquine and santonine J Clin Invest., 26 1168-72, 1947
- 151 JACQUES, L B AND LEPP, E Action of sodium salicylate on prothrombin time in rabbits. Proc. Soc. Exper Biol Med., 66: 178-81, 1947
- 152 JOHNSON, C C The salicylates XIX The question of acidosis following the administration of salicylates. J Am Med Assoc., 94 784-9, 1930
- 153 KAPP, E M Studies on the metabolism of sodium salicylate. Dissertation for Ph D Columbia Univ., 1942.
- 154 KAPP, E M AND COBURN, A F Urinary metabolites of sodium salicylate J Biol Chem., 145 549-565, 1942.
- 155 KASE, K Zur physiologischen Wirkung der Salicylsäurederivate, I Biochem Z., 205 21-6 1929
- 156 KEITH, J D AND HICKMAN, E M Vitamin C excretion in children with particular reference to rheumatic fever Arch. Diseases Childhood, 13 125-36, 1938
- 157 KEITH, J D AND ROSS, A Observations on salicylate therapy in rheumatic fever Can Med. Assoc J., 52 554-6, 1945.

153. KELLER J W, JR. A rapid method for the determination of salicylates in serum or plasma. *Am. J. Clin. Path.*, 17 415-7, 1947.
159. KRAKOFF S. O. AND BERNSTEIN, M. Acetylsalicylic acid poisoning with a report of a fatal case. *J. Am. Med. Assoc.*, 135 712-4 1947
160. KUTTER, L. AND RICHARDS, R. K. A new method for the evaluation of drugs affecting the reaction of mice to pain stimulation. *J. Lab. Clin. Med.*, 23 1535-40, 1943
161. LATHAM, P. W. Why does salicylic acid cure rheumatism? *Lancet* 1 1153-60, 1885
162. LAWSON, R. B. AND KAISER, A. D. Methyl salicylate poisoning. Report of a case of death in a child with rheumatic fever following the cutaneous administration of oil of wintergreen. *Arch. Pediat.*, 54 509-15, 1937
163. LEAS, R. D. Reducing substances in the urine. *J. Lab. Clin. Med.* 12 15-17, 1926
164. LEECH, C. B. The value of salicylates in prevention of rheumatic manifestation. *J. Am. Med. Assoc.* 95 932-4, 1930.
165. LEHMAN, A. J. Respiratory, inhibitory and paralytic effects of some barbital hypnotics and coal tar analgesics. *J. Pharmacol.*, 65- 235-42 1939
166. LEPPER, R. M. AND MILLER B. F. Personal communication.
167. LESTER, D. The non appearance of salicylates in the urine after the administration of dicumarol. *J. Biol. Chem.*, 154 305-6, 1944.
168. LESTER, D., LOLL, G. AND GREENBERG L. A. The fate of acetylsalicylic acid. *J. Pharmacol.*, 87: 323-42, 1943.
169. LEULIER, A., SOKIER, R. AND NOUVEL, G. Saliva parotidienne et salicylate de sodium. *Compt. rend. soc. biol.*, 140- 874-5 1946
170. LIGHT, J. A. AND HOOKER, S. P. Effect of acetyl salicylic acid on sedimentation rate of erythrocytes in rheumatic fever. *Proc. Soc. Exper. Biol. Med.*, 48: 69-70, 1941
171. LIGON, E. W., JR., MADDER, R. J., DAVIS, P. L. AND SMITH, P. K. Absorption of salicylates from intestinal loops in the dog. *Fed. Proc.*, 7 240, 1948
172. LITKE, K. P., OVERMAN, R. S., SULLIVAN, W. R., HUEBNER, C. F. AND SCHEEL L. D. Studies on the hemorrhagic sweet clover disease. XI. Hypoprothrombinemia in the rat induced by salicylic acid. *J. Biol. Chem.*, 147 463-74, 1943
173. LIPKOWITZ, S. Survival after 600 grains of aspirin. *Lancet*, 226 923 1934.
174. LIVON, C. Recherches sur l'action physiologique de l'acide salicylique sur la respiration. *Compt. rend.*, 90 321, 1880.
175. LOBERG, K. Eine klinische Methode zur quantitativen Bestimmung von Salicylsäure in Blutserum und Liquor cerebrospinalis. *Biochem. Z.*, 170 173-84, 1926.
176. LONCKECKER, H. Pharmakologische und klinische Untersuchungen zur Salicylsäuretherapie. *Z. ges. exper. Med.*, 93. 247-54, 1938
177. LOLL, G. AND SMITH, R. Effervescent mixtures as adjuvants to the rapid absorption of ingested drugs. *New Engl. J. Med.*, 235- 80-4, 1946.
178. LONGNECKER, H. E., FRISCKE, H. H. AND KING C. G. The effect of organic compounds upon vitamin C synthesis in the rat. *J. Biol. Chem.*, 135 497-510, 1940
179. LONGNECKER, H. E., MUGULY, R. R., TULLY, R. H., 3RD, AND KING, C. G. An acceleration of vitamin C synthesis and excretion by feeding known organic compounds to rats. *J. Biol. Chem.*, 129- 445-53, 1939
180. LOWENTHAL, J. AND GAGNON, A. The inhibition of hyaluronidase by sodium salicylate and its possible metabolites. *Can. J. Res. E.*, 26 200-5, 1943.
181. LOWY, O. A comparative study of the habitual use of barbiturates and coal tar derivatives as furnished by reports from various hospitals throughout the United States. *Can. Med. Assoc. J.*, 31 633-41, 1934
182. LUTWAK MANN, C. The effect of salicylate and cinchophen on enzymes and metabolic processes. *Biochem. J.*, 36 706-23 1942.
183. LUTWAK MANN, C. The excretion of a metabolic product of salicylic acid. *Biochem. J.*, 37 246-8, 1943
184. LUTWAK A. Recherches sur le mode d'action de l'acide salicylique et de l'acide p-aminosalicylique dans l'inhibition de la croissance du bacille tuberculeux. *Ann. Inst. Pasteur*, 76: 150-67, 1940
185. MACKEY, E. M., WICK, A. N., CARYL, H. O. AND BARNUM, C. P. The influence of alkalosis and acidosis upon fasting ketosis. *J. Biol. Chem.*, 138. 63-8, 1941.
186. MADISON, H. Über pathologisch-histologische Befund bei therapeutischer und experimenteller Natriumsalicylatvergiftung sowie deren günstige Beeinflussung durch Traubenzucker. *Deut. Arch. Klin. Med.* 176 612-23, 1934.
187. MACMONT, G. F. Some observations on salicylate therapy in children. *Biochem. J.*, 41 111, 1947
188. MANCHASTER, R. C. Rheumatic fever in naval enlisted personnel. II. Effectiveness of intensive salicylate therapy in cases of acute infection. *Arch. Internal Med.*, 78 170-80 1946
189. MANCHASTER, R. C. Rheumatic fever in naval enlisted personnel. III. The physiologic and toxic effects of intensive salicylate therapy in acute cases. *J. Am. Med. Assoc.*, 131 209-13, 1946
190. MANDL, A. R. Xanthin as a cause of fever and its neutralization by salicylates. *Am. J. Physiol.*, 29 432-43, 1907
191. MARTIN, G. J. AND BYERS, S. Biochemical studies of salicylic acid and a series of its derivatives. *Am. J. Digestive Diseases*, 15: 127-31 1943.
192. MASTER, A. M. AND ROMANOFF A. Treatment of rheumatic fever patients with and without salicylates. *J. Am. Med. Assoc.*, 93. 1979-80, 1932.
193. MAUGHAN, G. B. EVELYN, K. A. AND BROWNE, J. S. L. A method for the quantitative estimation of glucuronic acid and conjugated glucuronides. *J. Biol. Chem.* 176 597-72 1933

- 194 MCGUIGAN, H A AND HIGGINS, J A Some lesser known actions of the salicylates. *Arch. intern Pharmacodyn.*, 51 393-415, 1935
- 195 McLENNAN, C E, JACOB, B V AND MATSON, G A. Salicylates in the prevention of erythroblastosis fetalis. *Am J Med*, 3 661-6, 1947
- 196 MEYER, K AND RAGAN, C The antirheumatic effect of sodium gentisate. *Science*, 108, 231, 1948.
- 197 MEYER, K, RAGAN, C AND WEINHEILBAUM, H Inhibition of hyaluronidase by hydroquinones and quinones. *Fed. Proc.*, 7: 173-4, 1948
- 198 MEYER, O O AND HOWARD, B Production of hypoprothrombinemia and hypocoagulability of the blood with salicylates. *Proc. Soc. Exper Biol. Med*, 53 234-7, 1943
- 199 MIDDLEBROOK, G AND LLOYD, J B Effects of pH, temperature and salicylate on bacteriostasis of tubercle bacilli by sulfonamides and diaminodiphenylsulfone. *Am Rev Tuberc*, 49: 535-8, 1944
- 200 MONCORPS, C Untersuchungen über die Pharmakologie und Pharmakodynamik von Salben und salbeninhalierenden Medikamenten VII Mitteilung, Über die Resorption der Salizylsäure aus freie Salizylsäure, Salizylate und Salizylester enthaltenden Einreibemitteln (Antirheumatika) *Arch. exper. Path. Pharmacol.*, 143: 377-92, 1931
- 201 MORRIS, N AND GRAHAM, S The carbon dioxide dissociation curve of the blood in infancy and childhood. *Arch. Diseases Childhood*, 4 299-305, 1929
- 202 MORRIS, N AND GRAHAM, S The value of alkali in salicylate therapy *Arch. Diseases Childhood*, 6 273-84, 1931
- 203 MURATORE, F E PUTIGNANO, T L'azione dell'ac. p-aminobenzoico sulla concentrazione ematica e sulla eliminazione urinaria del salicilato di sodio. *Boll. soc. ital. biol. sper.*, 24 269-70, 1948
- 204 MURPHY, G E Salicylate and rheumatic activity, an objective clinical histologic study of the effect of salicylate on rheumatic lesions, those of joints and tendon sheaths in particular. *Bull. Johns Hopkins Hosp.*, 77: 1-42, 1945
- 205 MURRAY LYON, R M Salicylates in rheumatic fever. *Edinburgh Med J*, 43 84-93, 1935
- 206 News of the Industry, 1949 production of synthetic organic chemicals in U. S., *Chem. Eng. News* 27, 852 (1949)
- 207 OAKLEY, W AND DONNELLY, J P Massive aspirin overdosage. *Recovery* *Brit. Med. J.*, 787-9, 1942.
- 208 ODIN, M Is salicyl poisoning an "acidosis"? *Acta. med. Scand. (Suppl.)*, 50 177-86, 1932
- 209 OLIVESTAD, J G M AND ALDRICH, C A.: Acidosis in methyl salicylate poisoning. Report of two cases and a review of the literature. *J. Am. Med. Assoc.*, 90: 1438-40, 1928
- 210 OVERMAN, R S, STAUBMANN, M A, HUBBARD, C F, SULLIVAN, W R, SPERO, L, DORRERT, D G, IKAWA, M, GRAO, H, ROSEMAN, S., AND LINK, K P Hemorrhagic sweet clover disease, anticoagulant activity and structure in 4-hydroxycoumarin group. *J Biol Chem*, 153 5-24, 1944
- 211 OWEN, G C AND BRADFORD, H A The prothrombinopenic effect of massive salicylate therapy in acute rheumatic fever. *Ann. Internal Med.*, 25 97-102, 1945
- 212 PARKER, R A Factors influencing plasma concentrations of salicylate. *Quart. J. Med.*, 17 229-48, 1948
- 213 PARSONS, C M AND GOETTL, F R. The influence of acetylsalicylic acid upon analgesic effects of induced pain. *Permanent Foundation Med. Bull.*, 4 15-19, 1946
- 214 PAUL, F, WICK, A N AND MACKEAT, E M Inhibition of gastric ulceration in the rat by o-hydroxybenzoic (salicylic) acid. *Science*, 107 19-20, 1948
- 215 PAUL, W D The effect of acetylsalicylic acid (aspirin) on the gastric mucosa. A gastroscopic study. *J. Iowa State Med. Soc.*, 33 155-8, 1943
- 216 PELNER, L. The effect of ascorbic acid on the sensitivity of salicylates in a case of rheumatic fever. *J. Lab. Clin. Med.*, 28 28-30, 1942.
- 217 PERRY, C B The action of salicylates on the development of antibodies following anti typhoid inoculation. *J. Path. Bact.*, 53 291-3 1941
- 218 PETERS, J P AND VAN SLYKE, D D Quantitative clinical chemistry, vol 1 2nd ed., vii + 1041 pp, Williams and Wilkins, 1946
- 219 PINCOFFS, M C AND CHAMBERS, E T Methyl salicylate poisoning. *Bull. Maryland Sch. Med.* 17 57-60, 1932.
- 220 PRICKMAN, L E AND BUCHSTEIN, H F Hypersensitivity to acetylsalicylic acid (aspirin) *J. Am. Med. Assoc.*, 108 445-51, 1937
- 221 QUICK, A J The relationship between chemical structure and physiological response. I. The conjugation of substituted benzoic acids. *J. Biol. Chem.* 96 83-101, 1932
- 222 QUICK, A J The relationship between chemical structure and physiological response. II. The conjugation of hydroxy- and methoxybenzoic acids. *J. Biol. Chem.*, 97 403-19 1932
- 223 QUICK, A J The relationship between chemical structure and physiological response. IV. Conjugation of salicylic acid with glycine and its action on uric acid excretion. *J. Biol. Chem.*, 101 475-85, 1933
- 224 QUICK, A J The anticoagulants effective *in vivo* with special reference to heparin and dicumarol. *Physiol. Rev.*, 24: 297-313, 1944
- 225 RAPOPORT, S AND GUEST, G M The effect of salicylates on the electrolyte structure of the blood plasma. I. Respiratory alkalosis in monkeys and dogs after sodium and methyl salicylate. the influence of hypnotic drugs and of sodium bicarbonate on salicylate poisoning. *J. Clin. Invest.*, 24 759-69 1945
- 226 RAPOPORT, S AND GUEST, G M Effect of salicylate on plasma fibrinogen and sedimentation rate in rheumatic and non rheumatic patients. *Proc. Soc. Exper Biol. Med.*, 61 43-5, 1946
- 227 RAPOPORT, S, WING, M AND GUEST, G M Hypoprothrombinemia after salicylate administration in man and rabbits. *Proc. Soc. Exper Biol. Med.*, 53 40-1, 1943
- 228 REID, J Does sodium salicylate cure rheumatic fever? *Quart. J. Med.*, 17: 139-5, 1948.

- 229 RINEHART, J F Studies relating vitamin C deficiency to rheumatic fever and rheumatoid arthritis experimental, clinical and general considerations. *Ann. Internal Med.* 9 588-97 1935
- 230 ROBBINS, M L, STIBRAVY, L G, BOURKE, A R AND SMITH, P K. Unpublished
- 231 ROBERTS, R. C., CROCKETT, K A AND LAFFLY, T C Effects of salicylates and benadryl on experimental anaphylactic hypersensitive vascular and cardiac lesions. *Arch. Internal Med.* 83 48-68, 1949
- 232 ROCKWOOD, E W, The influence of the isomers of salicylic acid on metabolism. *Am J Physiol.* 25 34-42, 1909
- 233 ROCKWOOD, E W AND VAN EPPS, C The influence of some medicinal agents on the elimination of uric acid and creatinin. *Am J Physiol.* 19 97-107 1907
- 234 ROSEMAN, S, PEARSON, F AND DORFMAN, A *In vitro* action of gentisic acid on hyaluronidase. *Fed. Proc.* 8 245, 1949
- 235 ROSKAM, J Observations cliniques concernant le traitement de la maladie de Bouilland par le salicylate de soude et l'acide acetylsalicylique. *Acta Clin Belg.* 3 305-11 1948
- 236 RYDER, H W, SHAYER, M AND FERRIS, E B, JR. Salicylism accompanied by respiratory alkalosis and toxic encephalopathy. Report of a fatal case. *New Engl. J. Med.* 232 617-21, 1945
- 237 SALABA, R M, BOLLMAN, J L AND DRY, T J The effect of para-aminobenzoic acid on the metabolism and excretion of salicylate. *J. Lab. Clin. Med.* 33 1393-1401, 1948
238. SALTMAN, A Fluorophotometric method for the estimation of salicylate in blood. *J. Biol. Chem.* 174 399-404, 1948.
- 239 SCHERLIS, S Salicylate therapy in rheumatic fever. *J. Am. Med. Assoc.* 138 1253, 1948
- 240 SCHROEDER, F M AND JOHNSON, F H The influence of temperature and hydrostatic pressure on the denaturation of methemoglobin by urthanes and salicylate. *J. Biol. Chem.* 178 251-7, 1949
- 241 SCHNEEDORF, J G, BRADLEY, W B AND LYT, A C Effect of prolonged administration of salicylate upon nitrogen metabolism and plasma carbon dioxide combining power in the dog. *Am J Digestive Diseases*, 3 332-4, 1936
242. SCOTT, R W, THOBURN, T W AND HANZLIK, P J The salicylates. IV Salicylates in the blood and joint fluid of individuals receiving full therapeutic doses of the drug. *J. Pharmacol.* 9 217-25, 1917
243. SHAPIRO, S, REDISH, M H AND CAMPBELL, H A Studies on prothrombin. The prothrombinopenic effect of salicylate in man. *Proc. Soc. Exper. Biol. Med.* 53 251-4, 1943
244. SHOOKROFF, C AND LIEBERMAN, D L. Hypersensitivity to acetyl salicylic acid expressed by an angina pectoris syndrome with and without urticaria. *J. Allergy*, 4 500-12 1933
245. SMITH, P K, BATLIS, J R, ORGOZALEK, S AND MCCLURE, M M Metabolism of large doses of para aminobenzoic acid. *Fed. Proc.* 5 154-5 1946
246. SMITH, P K, GLEASON, H L, STOLL, C G AND ORGOZALEK, S. Studies on the pharmacology of salicylates. *J. Pharmacol.* 87 237-65, 1946
247. SMITH, P K, HAND, H A AND MADDEN, R. J The hydrolysis of acetylsalicylic acid by animal tissues. *Fed. Proc.* 6 373, 1947
248. SMULL, K., WEGRIA, R. AND LELAND, J The effect of sodium bicarbonate on the serum salicylate level during salicylate therapy of patients with acute rheumatic fever. *J. Am. Med. Assoc.* 125 1173-5, 1944.
249. SMULL, K., WISSEK, R. W. AND WARSON, J M The effect of sodium salicylate upon serum disease in rabbits. *J. Lab. Clin. Med.* 33 936-50, 1948
250. SMYTH, E S AND WHIFFLE, G H Bile salt metabolism IV Negative influence of drugs: atropine, pilocarpine, phlorizin quinine, etc. *J. Biol. Chem.* 59 655-9 1924
251. STANLEY, P G AND ALPERSON, C M Antagonism of salicylate by pantoyletaurine. *Science*, 103 398-9, 1946
252. STEVENS, D L AND KAPLAN, D B Salicylate intoxication in children. Report of four cases. *Am J Diseases Children*, 70 331-5, 1945.
253. STOCKMAN, R. The formation and action of salicyluric acid in the human body. *Edinb. Med. J.* 20 103-8, 1906.
254. SULLIVAN, C E J, PARKER, T W AND HERBERT, R. W Prevention by sodium salicylate of arthritis in the experimental allergic state. *Proc. Soc. Exper. Biol. Med.* 67 508-11, 1945
255. SWIFT, H F The action of sodium salicylate upon the formation of immune bodies. *J. Exper. Med.* 36: 735-60, 1922.
256. TAINTER, M L, TAINTER, E G, LAWRENCE, W S, NEURG, E N, LACKEY, R. W, LUDUENA, F P, KIRTLAND, H. B AND GONZALEZ, R. I Influence of various drugs on the threshold for electrical convulsions. *J. Pharmacol.* 79 42-53, 1943
257. TARAN, L. M. AND JACOBS, M. H. Salicylate therapy in rheumatic fever in children. *J. Pediat.* 27: 59-83 1945
258. TATYAU, F ET NEUHL, E Action du salicylate de sodium sur le sérum sanguin *in vitro* et *in vivo*. *Compt. rend. soc. biol.* 141 79-80, 1947
259. TAYLOR, H. M. Further observations on prenatal medication as a possible etiologic factor of deafness in the new born. *Southern Med. J.* 28 125-30 1935.
260. TERRELL, E H. AND SHAW, F W Observations of fungal infections of peri-anal skin and rectum. *Southern Med. J.* 21 587-9, 1928
261. THILLY, C H. Altered responses of smooth muscle to autonomic drugs produced by physical and chemical changes. *Arch. Intern. Pharmacodyn.* 31: 447-502, 1926
262. THOBURN, T W AND HANZLIK, P J The salicylates. II. Methods for the quantitative recovery of salicyl from urine and other body fluids. *J. Biol. Chem.* 23 163-80 1915.
263. THOMAS, W C AND STRINGFIELD, C Effect of maintaining high salicylate levels on the lesions of rabbits with serum sickness. *Proc. Am. Fed. Clin. Res.* 2 83-4, 1945
264. THOMPSON, H E AND DRAGSTEDT, C A Modifying action of calcium and sodium bicarbonate on salicylate intoxication. *Arch. Internal Med.* 54: 303-12, 1934.

- 265 VANDELLI, I, AND SCALTRIT, F Alcuni organi animali sono ricchi di un fermento, che è capace di scindere, acetilsalicilico, che non si può identificare con l'acido collino-esternale e che viene ad accrescersi ripetute di aspirina. Boll. soc. ital. biol. sper., 18 77-9, 1943
- 266 VAN EELKEN, M Effect of acetylsalicylic acid on urinary excretion of ascorbic acid. Acta brev. neerl. 7 69-70, 1937
- 267 WADDELL, J A A comparative investigation of the effects and toxicity of sodium salicylates of natural and synthetic origin. Arch. Internal Med., 8 784-803, 1911
- 268 WARREN, H A, HIOLET, C S AND COOMBS, F S The effect of salicylates on acute rheumatic fever. Am. Heart J. 32 311-26, 1946
- 269 WATKINSON, G Massive salicylate therapy in rheumatic fever. Ann. Rheumatic Diseases, 8 120-4, 1949
- 270 WEFORIA, R AND SMULL, K Salicylate therapy in acute rheumatic fever. J. Am. Med. Assoc., 129 435-90, 1945
- 271 WEFORIA, R AND SMULL, K. Salicylate therapy in acute rheumatic fever. J. Pediat., 26 211-3, 1945
- 272 WEICHELBAUM, T E AND SHAPIRO, I A rapid and simple method for the determination of salicylic acid in small amounts of blood plasma. Am. J. Clin. Path., 15 42-4, 1945
- 273 WHITEHEAD, H C Comparative action of sulfanilamide, sulfapyridine, sulfathiazole and acetylsalicylic acid (aspirin) on growth of the tubercle bacillus *in vitro*. J. Thoracic Surg., 10 372-5, 1941
- 274 WIKLER, A, GOORELL, H AND WOLFF, H G Studies on pain. The effects of analgesic agents on sensations other than pain. J. Pharmacol., 83 284-9, 1945
- 275 WILLIAMS, F AND LEONARD, J R The effect of sodium bicarbonate on the renal excretion of salicylate. J. Pharmacol., 93: 401-8, 1948
- 276 WILLIAMS, S W AND PANTINO, R M Treatment of aspirin poisoning by intravenous sodium lactate solution. Brit. Med. J., 550-2, 1937
- 277 WILLIAMSON, J AND RUNGE, E A Antipyretic action and catalase activity. Biochem. J., 43 15-16, 1948
- 278 WINDER, C V Quantitative evaluation of analgesic action in guinea pigs. Morphine, ethyl 1-methyl-4-phenyl piperidine-4-carboxylate (Demerol), and acetylsalicylic acid. Arch. Intern. Pharmacodyn., 74, 219-32, 1947
- 279 WINTER, J E AND BARBOUR, H G Influence of magnesium salts upon the toxicity and antipyretic action of salicylates. Proc. Soc. Exper. Biol. Med., 25 587-90, 1928
- 280 WOLFF, H G, HARDT, J D AND GOORELL, H Measurement of the effect on pain threshold of acetyl salicylic acid, acetanilid, acetophenetidin, aminopyrine, ethyl alcohol, trichlorethylene, a barbiturate, quinine, ergotamine tartrate and caffeine: an analysis of their relation to pain experience. J. Clin. Invest., 20 63-80, 1941
- 281 WRIGHT, S The mode of action of certain drugs which stimulate respiration. J. Pharmacol., 54 1-16, 1933
- 282 YOUNG, J B, CORLETTE, M B, FRANK, H AND CORLETTE, M Failure of acetylsalicylic acid to affect excretion of ascorbic acid (vitamin C) in urine. Proc. Soc. Exper. Biol. Med., 36 73-8, 1937
- 283 YOUNGVER, J S, AND ALTSCHULER, C H Failure to relate hyaluronic acid to elevated erythrocyte sedimentation rate in rheumatic diseases. Proc. Soc. Exper. Biol. Med., 67 92-6, 1946
- 284 YU, T F AND GUTMAN, A B Interference of gentisic acid in determination urinary uric acid after administration of salicylates. Fed. Proc. 8: 267, 1949



# MONOFLUOROACETIC ACID AND RELATED COMPOUNDS<sup>1</sup>

MAYNARD B CHENOWETH

*Department of Pharmacology, University of Michigan, Ann Arbor, Michigan*

During and since World War II there has been a widening interest in the polyfluorinated hydrocarbons, most of which are very nearly inert biologically. On the other hand, a group of monofluorinated compounds has been found that includes many very toxic substances. Monofluoroacetic acid is the prototype of these compounds, which exhibit pharmacological actions of remarkably different character in different species. It is the purpose of this review to bring together and attempt to explain the available information concerning these potent pharmacological agents. The review has not been undertaken primarily because of interest in the fluoroacetates<sup>2</sup> *per se* but rather because their study contributes greatly to the pharmacological and biochemical understanding of drug actions.

Although first prepared synthetically by Swarts (122) in 1896, monofluoroacetic acid ( $\text{FCH}_2\text{COOH}$ ) and its derivatives attracted very little attention from chemists and none from pharmacologists until the early 1940's when Polish chemists (96), escaping to England, brought word of the toxicity of the methyl ester of fluoroacetic acid which they had prepared (60). It was soon established by intensive studies under the auspices of the armed services in England and in this country that the fluoroacetates are exceedingly curious substances. The compounds are so toxic to dogs that 50 micrograms per kilogram cause prolonged convulsions of central nervous system origin and death from respiratory failure, yet they are only 1/200 as toxic to monkeys, in which species cardiac poisoning and ventricular fibrillation are the cause of death. The reasons for these seemingly capricious effects present problems of interest to biologists.

Security precautions during the war prevented prompt publication of research results and it therefore occasionally was difficult to acknowledge properly some of the investigative work done during that period. During this period Marais, in an independent study reported in 1944 (93), succeeded in isolating potassium monofluoroacetate from the South African plant, *Dichapetalum cymosum*. This plant, known locally as "Gifblaar," is a well-recognized hazard to stock and cattle (131). The occurrence of fluoroacetic acid in this plant is believed to be the first example of a naturally occurring organic fluoride, it completes the list of natural organic halogen compounds and presents an interesting problem in biosynthesis.

A considerable body of practical experience with the use of sodium fluoroac-

<sup>1</sup> Original investigations reported in this communication were supported (in part) by research grants from the Division of Research Grants and Fellowships of the National Institutes of Health, U S Public Health Service.

<sup>2</sup> The use of "fluoroacetates" as a loose generic name for this series of compounds is based on the fact that fluoroacetic acid and its simple compounds are the lowest active members of a homologous series.

tate (Compound # 1080, hence the common name of "ten eighty") as a rodenticide and general mammalian pest control agent has accumulated since Treichler and Ward of the United States Fish and Wildlife Agency introduced the compound for this purpose in 1945. The pertinent original literature should be consulted by those interested in this important application of fluoroacetate (5, 6, 66, 74, 92, 119, 128). It will be reviewed here only when information it contains contributes to the understanding of the biological actions of these compounds. The rodenticidal uses of the chemical cannot be ignored for such uses are of prime ecologic importance. The animal population of a poisoned area may be almost entirely destroyed by sodium fluoroacetate and its widespread use in pest control has been the cause of several human fatalities.

## I SPECIAL CHEMISTRY OF THE FLUOROACETATE COMPOUNDS

### A Synthesis

The synthesis of fluoroacetic acid, its derivatives and analogs has been studied intensively (60, 93, 122), particularly with the objective of perfecting large scale processes (7, 24, 72, 110, 111, 112, 113). Jenkins and Koehler have described the process and safeguards employed by the chief manufacturer of "1080", sodium fluoroacetate (73). Germane to the present review is the fact that most commercial procedures for production of "1080" result in some contamination of the product with fluorides (usually KF) unless special effort, usually redistillation of an ester of fluoroacetic acid, is taken to purify the product. This can be a source of error in interpreting the results of studies with fluoroacetate, particularly with high concentrations *in vitro*.

### B Structural Aspects

Certain details concerning the physical chemistry of fluoroacetate are important for an understanding of its pharmacological actions. The chemical behavior of monofluorinated organic acids in general is very little different from that of the corresponding unsubstituted acids, this is in contrast to the other monohalogenated acids. Indeed, the process of fluorination, with the corresponding shortening and strengthening of inter-atomic bonds, appears to confer increasing nonreactivity on all molecules (61).<sup>\*</sup>

Attention has been directed particularly to the stability of the carbon-fluorine bond in fluoroacetic acid. There are little or no data upon the nature of this bond in longer-chain acids but their pharmacological behavior suggests that the stability of their C-F bond parallels that in fluoroacetate. It has been confirmed repeatedly that rupture of the C-F bond is very difficult (12, 13, 40, 110). Although some samples of sodium fluoroacetate do contain fluorides as contaminants, it is not reasonable to suppose that the fluoroacetates exert their actions by the liberation of fluoride, the pharmacological actions of which are quite different (108). Indeed, Bergmann and Fruton found that fluoroacetate, even after many

<sup>\*</sup> The chemistry of the aliphatic fluorine compounds, as it was known in 1941, has been reviewed by Henne (61). Two additional recent reviews (14, 101) of the expanding field of fluorine chemistry should be consulted by those interested.

hours in solution under physiological conditions of pH and temperature, loses no fluorine detectable as fluoride ion (13). However, boiling with 20 per cent KOH for 20 hours will release 50 per cent of the fluorine as KF (110). Bartlett and Barron have published a large list of biologically important chemical substances and enzymes with which fluoroacetate does *not* react, and have contrasted the four monohalogenated acetic acids with regard to their ability to thioacetylate cysteine (12) (see Table I). The rate of replacement of the fluorine of ethyl fluoroacetate by sulfite at 45° C is expressed by a bimolecular velocity constant of  $4.5 \times 10^{-5}$ , whereas the bromine of ethyl bromoacetate is replaced at 25°C at a very much greater rate (constant = 18.3), according to Backer and van Mels (8). This extreme nonreactivity of carbon-attached fluorine reflects the general relationship between the rates of reaction and the bond-energy values of the C-halogen bonds as well as the electro-negative values of the halo-

TABLE I

*Physical and Chemical Properties of Acetic Acid and Monohalogenated Acetic Acids*  
(References to source of data are given in parentheses)

COMPOUND	DIS- SOCIATION CONSTANT OF ACID $K_a \times 10^{-4}$ (130)	ATOMIC RADIUS (104)	INTER- NUCLEAR DISTANCE IN ÅNG- STROMS (115)	BOND ENERGY IN KILO-CAL- ORIES PER MOLE (103)	HALOGEN ELEC- TRONEG- ATIVITY (103)	REACTION RATE WITH CYSTEINE. TIME TO HALF REACTION AT 25 C. (12)	LD <sub>50</sub> MICE Na SALT ORALLY
HCH <sub>2</sub> COOH	1.8	(H) 0.29	1.14	87.3	—	—	mgm/kgm
FCH <sub>2</sub> COOH	210	(F) 0.64	1.45	107.0 114 (59)	4.0	No reaction	17(125)
ClCH <sub>2</sub> COOH	155	(Cl) 0.99	1.74	66.5	3.0	125 mins	165(100)
BrCH <sub>2</sub> COOH	138	(Br) 1.14	1.90	54.0	2.8	6.2 mins	100(100)
ICH <sub>2</sub> COOH	75	(I) 1.33	2.12	45.5	2.5	4.0 mins	63(100)

gens (Table I). It may be noted that, although chloroacetic acid is the strongest acid of the Cl, Br, I-monohalogenated acetic acids, iodoacetic is the most toxic and chloroacetic the least toxic. The toxicity is probably a reflection of their relative activity as thiol acetylating agents. But fluoroacetic acid, the strongest monohalogenated acetic acid, is also the most toxic. It is apparent that toxicity in this series is directly related to the reactivity of the halomethyl, rather than the carboxyl, moiety of the molecule. The great difference in chemical character between fluoroacetic acid and the more familiar iodoacetic acid is almost certainly a direct cause of the equally great difference in pharmacological action.

### C Analytical Aspects

There appears to be no chemical reaction given solely by fluoroacetic acid which would distinguish it from other compounds. Reactions of acetic acid which depend upon attack of the methyl group do not proceed at all or only under much more drastic conditions when attempted with fluoroacetic acid,

although there is no appreciable qualitative difference with regard to reactions involving the carboxyl groups. Fluoroacetate solutions give the characteristic blue color given by acetate and propionate with lanthanum salts (Kruger and Tschirch test). Hutchens and Kass (68) have made this test quantitative under very controlled conditions, for example, it is capable of detecting fluoroacetate in culture media in a range of 100 to 400 parts per million. The crystalline forms of the barium salts of the four monohalogenated acetic acids are useful for identification and have been described in detail (46). Characterization of sufficiently large amounts of pure fluoroacetic acid has been accomplished by the formation of conventional and suitable derivatives (60, 73, 110), especially fluoroacetamide (7), a useful intermediate.

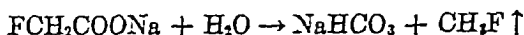
Although there appears to be no great difficulty in characterizing fluoroacetic acid in the organic chemist's laboratory, quite the contrary is true when determination of small amounts of the compound in biological material is attempted. To date, nearly all methods depend upon splitting off and detecting the fluoride ion. Quantitative methods for the determination of fluoride with zirconium alizarin sulfonate (57) or as lead chlorofluoride (49) have been employed. Much has been accomplished toward the detection of fluoroacetate in drinking water by drastic measures to release the fluoride ion which are rather convenient for field use (57). Ramsey and Clifford have recently described (106) a method for the accurate and specific determination of fluoroacetate in food and other biological material in concentrations as low as 0.2 part per million, a degree of sensitivity which has very practical importance.

#### *D Stability of Sodium and Methyl Fluoroacetate Solutions*

The extraordinary stability of the fluorine-carbon bond has attracted considerable attention and has led to the impression that compounds of fluoroacetic acid are very stable. This is not entirely true. Although solid sodium fluoroacetate, which is highly hygroscopic, keeps well in a desiccator, aqueous solutions of salt or esters decrease in toxicity with time. Albaum (2) showed that methyl fluoroacetate solutions decreased in toxicity even though refrigerated, while at room temperature the process was accelerated. Thus, a solution injected intraperitoneally in the calculated dose of 5 mgm/kgm killed 7 of 10 rats when it was fresh but only 2 of 12 rats after it was kept 24 days in the refrigerator (approximately 5°C). A second solution deteriorated at room temperature so markedly that, although 5 mgm/kgm killed 19 of 24 rats when injected immediately after being prepared it killed but 8 of 20 rats after it had stood for only 7 days. Although precise assays have not been made, observations in this laboratory with solutions of sodium fluoroacetate and sodium  $\gamma$ -fluorocrotonate have indicated a similar rate of deterioration. As far as toxicity to yeast is concerned, however, fluoroacetate solutions remain unchanged for 1 month at 3° to 5°C (77).

It is to be expected that hydrolysis of the methyl ester rapidly occurs and Price and Jackson (102) have found a half-life of less than an hour for this reaction at pH 7.0. This is not particularly important as a cause of the decreasing

activity inasmuch as a similar decrease is noted with the sodium salt. As pointed out under section I-B, no fluoride ion is released under these conditions. Woods (133) has suggested the possibility that spontaneous decarboxylation accelerated by the resonance effects of the fluorine atom may occur according to the reaction



with the liberation of the highly volatile, relatively non-toxic methyl fluoride. Thus, although no fluoride ion would accumulate in the solution, the actual content of fluoroacetate in the solution continually decreases. This type of reaction is often catalyzed by traces of halogen ions. Until more is known about this phenomenon, investigators using fluoroacetates should prepare solutions just prior to use. Deterioration does not seem to be a serious problem when solutions of sodium fluoroacetate are used in routine rat poisoning operations, perhaps because of the generally short (2 to 3 day) period of exposure of the poison (6).

## II TOXICITY

### A Response of Various Species to Toxic Monofluorinated Acids

Very few compounds are known which exert such variable pharmacological actions in different species as does fluoroacetate. Not only does the  $\text{LD}_{50}$  vary from 0.06 mgm/kgm in the dog to well over 500 mgm/kgm in the unique case of the South African clawed toad (*Xenopus laevis*), but the qualitative character of the pharmacodynamic action of the drug is equally varied. The major point of attack may be either the central nervous system or the heart. Both may be affected to varying degrees in some species, but it is usual to find that one organ is primarily concerned while the other is but slightly, or not at all, affected. Death may result from (a) respiratory arrest following severe convulsions, (b) gradual cardiac failure or ventricular fibrillation, or (c) progressive depression of the central nervous system with either respiratory or cardiac failure as the terminal event. All these responses follow a long and essentially irreducible latent period after the administration of the poison by any route. These phenomena are discussed in section III.

In Table II, the main toxic effect, if known, is indicated by 4+ and on the same scale 1+ indicates that this effect is very rarely seen. Although there is some difference between the sodium salt and the methyl ester of fluoroacetic acid when they are applied to frog tissue (18, 23, 38, 39), there appears to be no difference between them in the intact animal (30). No distinction between the two chemicals is made in Table II although most of the data were obtained with sodium fluoroacetate.

Study of the information presented in Table II indicates that, among the warm-blooded species, primates and all types of birds are generally the least susceptible to fluoroacetate poisoning, whereas the carnivora and wild rodents appear to be particularly sensitive. The Texan pocket gopher (*Geomys breviceps* sp.) is the most sensitive species so far described, all nine of those studied being killed by an intraperitoneal injection of 0.05 mgm/kgm. It is noteworthy that

TABLE II  
Toxicity of Fluoroacetate Compounds

SPECIES	LD <sub>50</sub>	ACCURACY*	ROUTE	HEART†	BRAIN†	REFERENCE
Sodium or methyl fluoroacetate, fluoroethanol						
	mgm./kgm.					
PRIMATES						
Man ( <i>Homo sapiens</i> )	2-5	3	Oral	4+VF, F	2-3+C	6
Rhesus Monkey ( <i>Macaca mulatta</i> )	40	2	I V	4+VF, F	2-3+C	30
Spider Monkey ( <i>Ateles geoffroyi</i> )	150	2	I V	4+VF, F	0	30
UNGULATES AND RUMINANTS						
Goat	0.6	2	I.M	4+VF	0	30
Sheep	20	3	Oral	4+VF	0	105
Horse	10	2	Oral	4+VF	1+C	30, 52, 53
Swine (young)	0.4	1	I.P	4+VF	4+C	30
Swine (adult)	<10	2	Oral	4+VF	4+C	115
CARNIVORES						
<i>Canines</i>						
Dog (mixed breeds)	0.08	2	I V	0	4+C	30
	0.10 (LD <sub>100</sub> )	1	I V	0	4+C	30, 37
Coyote ( <i>Canis latrans nebrascensis</i> )	0.10	1	I P	0	4+C	129
FELINES						
Cat ( <i>Felis domesticus</i> )	0.20	1	I V	2+VF	4+C	99
RODENTS						
<i>Rats</i>						
Albino Rats	50	1	I.M S C I P	1+VF 2+F	3+C 4+D	30, 129
	2-3	2	S C	—	—	52
	2.5	—	Oral	—	—	74
Cotton Rat (Florida) ( <i>Sigmodon hispidus littoralis</i> )	0.1	1	Oral	—	—	129
Wood Rat (California) ( <i>Neotoma intermedia</i> )	1.5	2	Oral	—	—	129
Wood Rat (Arizona) ( <i>Neotoma a. albigula</i> )	0.8	1	I P	—	—	129
Norway (Adult Wild Maryland) ( <i>R. R. Norvegicus</i> )	0.22	1	Oral	—	—	44
Norway Rat (Florida)	30	2	Oral	—	—	129
Alexandrine Rat ( <i>R. r. alexandricus</i> )	0.5	3	Oral	—	—	129
Black rat ( <i>R. r. subsp.</i> )	0.1	—	Oral	—	—	74
<i>Mice</i>						
Albino (Maple Grove)	19.3	1	S C	0	4+C	70
(Carworth)	17.0	1	Oral	—	—	125

TABLE II—Continued

SPECIES	LD <sub>50</sub>	ACCURACY*	ROUTE	REACT†	REACT‡	REFERENCE
Sodium or methyl fluoroacetate, fluoroethanol—Continued						
	mgm./kgm.					
<i>Mice—Cont'd</i>						
Albino others	10 0	1	I.P.	—	—	129
	16 0	2	S.C.	—	—	105
	5 0	2	S.C.	—	—	52
Meadow Mouse ( <i>Microtus haydeni</i> )	0 5	2	Oral	—	—	129
Deer Mouse ( <i>Peromyscus</i> sp.)	4 0	1	Oral	—	—	129
House Mouse ( <i>Mus musculus</i> )	8 0	2	Oral	—	—	129
Hamsters	3 0	2	I.P.	—	3+C 4+D	30
<i>Rabbits</i>						
New Zealand White	0 25	1	I.V.	4+VF	0	30, 70
Pigmented	0 5	3	I.V.	4+VF	0	37
Dutch & others	0 5-1 0	3	S.C.	—	—	30, 70
<i>Guinea Pigs</i>						
	0 35	1	I.P.	0	4+C	30, 70
	0 25 (LD <sub>100</sub> )	2	S.C.	—	—	105
<i>Ground Squirrels</i>						
Apache spotted ( <i>Citellus spilosoma caevescens</i> )	0 4	3	I.P.	—	—	129
Columbian ( <i>Citellus columbianus columbianus</i> )	0 9	2	I.P.	3+	4+D	129
Fisher ( <i>Citellus b. fisheri</i> )	0 3	—	Oral	—	4+C	129
<i>Pocket Gopher</i>						
Breviceps—Texas ( <i>Geomys breviceps</i> sp.)	<0 05 (LD <sub>100</sub> in 9 animals)		I.P.	—	—	129
Tuza—Florida ( <i>Geomys floridanus</i> )	0 2	2	I.P.	—	—	129
<i>Kangaroo Rats</i>						
Bannertail ( <i>Dipodomys spectabilis</i> )	0 1	1	I.P.	—	—	129
Merriam ( <i>Dipodomys merriami</i> )	0 15	2	I.P.	—	—	129
<i>BIRDS</i>						
<i>Chickens</i>						
White Leghorn	7 5	1	Oral	—	3+D	42
Rhode Island Red	5 0	3	Oral	4+	3+D	30, 129
			I.V.			
Plymouth Rock	5 5	2	Oral	—	—	129

TABLE II—Continued

SPECIES	LD <sub>50</sub>	ACCURACY*	ROUTE	HEART†	BRAIN†	REPER- ENCE
<i>Pigeons</i>	mgm /kgm					
Florida	9 0	2	Oral	—	—	129
Colorado	2 5	2	Oral	—	—	129
<i>Passerine</i>						
English Sparrow ( <i>Passer domesticus</i> )	2 5	1	Oral	—	—	129
<i>Game Birds</i>						
Gambels Quail ( <i>Lophortyx gambeli</i> )	20	1	Oral	—	—	129
<i>Carrion Feeding Birds</i>						
Golden Eagle ( <i>Aquila chrysaetos</i> )	5 0	3	Oral	—	—	129
Black vulture ( <i>Cathartus urubus</i> )	15 0	3	Oral	—	—	129
POIKILOOTHERMS						
<i>Frog</i>						
<i>Rana pipiens</i>	150 0	1	S C	0	2+C 4+D	30, 99
South African Clawed Toad ( <i>Xenopus laevis</i> )	>500 0	2	I P S C	—	—	105
Sodium $\gamma$ -fluorocrotonate $\text{FCH}_2\text{CH}=\text{CHCOONa}$						
Rhesus Monkey ( <i>Macaca mulatta</i> )	2 5	3	I V	4+VF	2+C	37
Dog	0 05-0 07	2	I V	0	4+C	37
Rabbit (Albino)	0 15	2	I V	4+VF	0	37
Mouse (Albino) (Rockland Swiss)	1 0	2	I V	0	4+C	37
	2 0 (LD <sub>100</sub> )	1	I V	0	4+C	37
Rat (Albino)	1 0	2	I P	0	3+C 4+D	37
<i>Rana pipiens</i>	25 0	3	S C	—	2+C 4+D	37
Methyl $\gamma$ -fluorobutyrate $\text{F-CH}_2\text{CH}_2\text{CH}_2\text{COO CH}_3$						
Rhesus Monkey ( <i>Macaca mulatta</i> )	3-5	3	I V	4+F	3+C	29
Cat	0 2	2	I V	0	4+C	29
Rabbit	0 10	1	I V	4+VF	1+C	29



TABLE II—*Concluded*

SPECIES	LD <sub>50</sub>	ACCURACY*	ROUTE	HEART†	BRAIN†	REFERENCE
Ethyl 5-fluorohexanoate F-CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> COO C <sub>2</sub> H <sub>5</sub>						
	mgm./kgm.					
Rabbits	0 2-0 5	2	I V	—	—	109
Rats	2 3	2	I.M	—	—	109
Mice	4 0	2	S C	—	—	109

Key to Scale — = No information

0 = Never seen.

1+ = Very rarely seen

2+ = Occasionally seen Can be expected

3+ = Generally seen

4+ = Characteristic action of drug Always seen

\* Accuracy 1—Highly accurate

2—Accurate enough for practical work

3—Estimate on few observations

† VF = Ventricular fibrillation

F = Cardiac failure (not VF)

C = Convulsion

D = Depression

See (96, 109, 129) for data on a number of other species and compounds which were not suitable for inclusion in this table

laboratory strains of rats and mice are quite resistant to fluoroacetate, but that there is much variation between strains (see also section III-F, d) There is a tendency, to which the guinea pig is a striking exception, for herbivorous animals to manifest cardiac effects and for carnivores to develop *per primum* central nervous system convulsions or depression, whereas in more or less omnivorous species both the heart and central nervous system may be affected Although a sex difference in respect to sensitivity has been reported for certain wild ducks (129), it has not been noted in other species As might be expected, elevated environmental temperatures increase the sensitivity of mice to fluoroacetate to an appreciable degree (37)

Cold-blooded vertebrates are generally very insensitive to fluoroacetate, but it is clear that the increased toxicity of fluorocrotonate is much more apparent in *Rana pipiens* than in most mammals The sensitivity of frogs to fluoroacetate is not significantly increased when the frogs are kept in water at 32°C (37), but frogs are more sensitive in the summer than in the winter (18)

Such data as are available indicate that fish are relatively insensitive to fluoroacetate in the water surrounding them (81) *Anopheles larvae* are very sensitive to fluoroacetate (43) Indeed, the insects that have been studied (ants, roaches (5), aphids (65) and moths (72)) are generally very sensitive to fluoroacetate Fleas are killed by feeding on poisoned rats (92) Microorganisms have not been studied extensively, but Kalnitsky and Barron (77) have made considerable use

of sensitive microorganisms and plant seedlings in elucidating the mechanism of action of fluoroacetate (v1) Mold growth (*Physarella Oblonga* Morgan) is inhibited by fluoroacetate in low concentrations(1) As a matter of practical importance Gratch and coworkers (58) have reported that fluoroacetate in concentrations used for rat poisoning (approximately 2%) has no bacteriostatic properties against *P. pestis* Therefore, it does not interfere with cultivation of these organisms from the tissues of rats obtained by poisoning operations with fluoroacetate

### *B Active and Inactive Fluorinated Compounds*

As various monofluorinated compounds, almost exclusively aliphatic, were prepared and tested for toxicity, it became apparent that slight changes in structure were sufficient to abolish completely the dramatic fluoroacetate-like activity In Table III the structures of a number of key compounds have been arranged under two columns "active" and "inactive" (An "inactive" compound is one which has little pharmacological activity, or no more than might be expected from the corresponding non-fluorinated compound, for example, 50 mgm /kgm of 1-fluoropropanol have no effect on rabbits (see footnote 3) Certain conclusions can immediately be made when such a tabulation is inspected The only active compounds are straight-chain compounds with an *even number* of carbon atoms in which *one fluorine atom* is substituted in a *terminal position* However, certain other requirements must be met Thus,  $\text{FCH}_2\text{CH}_2\text{Cl}$  is inactive although  $\text{FCH}_2\text{CH}_2\text{OH}$  is very active, presumably because little conversion of  $-\text{CH}_2\text{Cl}$  to  $-\text{COOH}$  can occur in the body, whereas fluoroethanol, analogously to unsubstituted ethanol, can be rapidly oxidized to the corresponding acetate in the body Fixation of the  $\alpha$  and  $\beta$  carbons of  $\gamma$ -fluorobutyrate in a methylene ring or loading the  $\beta$  carbon of  $\gamma$ -fluorobutyrate with one or two methyl groups results in inactivity On the other hand,  $\gamma$ -fluoro  $\beta$ -hydroxy butyrates are very active The conclusion is inescapable that compounds in the series which cannot form the fluoroacetate ion directly or through biochemical alteration have no characteristic fluoroacetate activity This conclusion was understood in its essential features by McCombie and Saunders as early as 1943 (96)

It does not necessarily follow, however, that the toxicity of compounds capable of forming fluoroacetate *in vivo* is due *entirely* to the formation of fluoroacetate There is evidence that  $\gamma$ -fluorobutyrate, for example, exerts a toxic action *per se*, independently of any action exerted by the fluoroacetic acid which may be formed by the  $\beta$ -oxidation of  $\gamma$ -fluorobutyric acid Indeed, the toxicity and pharmacodynamics of the two compounds are quite dissimilar, which would appear to be sufficient evidence for a difference in mechanism For example, progressive cardiac failure without ventricular fibrillation is noted in rhesus monkeys poisoned with fluorobutyrate, and fluorobutyrate-poisoned rabbits manifest signs of parasympathetic stimulation which are not characteristic of fluoroacetate (29) In addition, Kalnitsky and Barron have shown that rabbit kidney cortex, at least, does not convert fluorobutyrate to fluoroacetate and that the effects of the two agents are distinctly different (78)

TABLE III  
Active and Inactive Compounds  
(Data from 24, 25, 96, 108, 109, 110, 111, 112, 113)

NUMBER OF CARBONS IN LONGEST STRAIGHT FLUORINATED CHAIN	ACTIVE	INACTIVE
1	None	F-COO C <sub>2</sub> H <sub>5</sub> F-CH <sub>3</sub>
2	$\text{FCH}_2\text{COO} \begin{cases} \text{Salts} \\ \text{Esters} \end{cases}$  FCH <sub>2</sub> CHO FCH <sub>2</sub> CH <sub>2</sub> OH FCH <sub>2</sub> CN FCH <sub>2</sub> CONH <sub>2</sub> FCH <sub>2</sub> COF FCH <sub>2</sub> COCI	$\begin{array}{c} \text{F} \\ \diagdown \\ \text{CHCOO} \begin{cases} \text{Salts} \\ \text{Esters} \end{cases} \\ \diagup \\ \text{F} \end{array}$  $\begin{array}{c} \text{F} \\ \diagdown \\ \text{CHCOO}^- \\ \diagup \\ \text{Cl} \end{array}$ FCH <sub>2</sub> CH <sub>2</sub> -O-CH <sub>2</sub> CH <sub>2</sub> COOH FCH <sub>2</sub> CH <sub>2</sub> Cl  ClCH <sub>2</sub> COF FCH <sub>2</sub> CH <sub>2</sub> SO <sub>2</sub> Cl
3	None	FCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> OH CH <sub>3</sub> CHF <sub>2</sub> COO CH <sub>3</sub>
4	$\text{FCH}_2\text{CH}_2\text{CH}_2\text{COO} \begin{cases} \text{Salts} \\ \text{Esters} \end{cases}$  FCH <sub>2</sub> CHOHCHCOO CH <sub>3</sub>  $\text{FCH}_2\text{CH}=\text{CHCOO} \begin{cases} \text{Salts} \\ \text{Esters} \end{cases}$	CH <sub>3</sub> CH <sub>2</sub> CHF <sub>2</sub> COO CH <sub>3</sub> $\text{FCH}_2-\text{CH}-\text{CH}-\text{COO CH}_3$ $\begin{array}{c} \text{HC} \quad \text{CH}_2 \quad \text{CH} \\ \diagdown \quad \diagup \quad \diagdown \\ \text{CH} \quad \text{CH} \end{array}$  $\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{FCH}_2-\text{CH}-\text{CH}_2\text{COO C}_2\text{H}_5 \\ \diagdown \\ \text{CH}_3 \end{array}$
5	None	FCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> COO R
6	FCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> COO R	
7	—	—
8	FCH <sub>2</sub> (CH <sub>2</sub> ) <sub>4</sub> COO R	—
9	—	—
10	FCH <sub>2</sub> (CH <sub>2</sub> ) <sub>6</sub> COO R	—
11	None	FCH <sub>2</sub> (CH <sub>2</sub> ) <sub>8</sub> COO R
12	FCH <sub>2</sub> (CH <sub>2</sub> ) <sub>10</sub> COO R	

On the other hand,  $\gamma$ -fluorocrotonic acid does not appear to have any qualitative action in the intact animal different from that of fluoroacetate. This may be related to an increased ease of oxidation at the double bond, so that fluorocrotonic acid perhaps exists as such for a very much shorter time than does fluorobutyric acid. The greater toxicity of fluorocrotonate may be related to the greater ease with which it penetrates to the active area. Fluoroethyl fluoroacetate ( $\text{FCH}_2\text{COOCH}_2\text{CH}_2\text{F}$ ) is about as active as fluoroacetate on an equimolar basis (111), but it acts much more quickly, this suggests an increased rate of cellular penetration (29).

Fluoroacetyl salicylic acid (110) may be cited as another example of a molecule which has an action not to be expected from the sum of actions of its groupings. It is as toxic to mice as fluoroacetate on a weight basis (compare acetylsalicylic acid), yet the characteristic action of fluoroacetyl salicylic acid is depressant rather than convulsant as would be expected if deacetylation with the liberation of fluoroacetate occurs in a manner similar to that described by Smith (120) for acetylsalicylic acid.

It appears that a fluorinated compound must have a structure capable of partially, but *not completely*, mimicking a natural metabolite if it is to be highly active pharmacologically. Factual evidence favoring this attractive interpretation of the data of Table III will be presented in Section IV. The serious consequences of such imperfect mimicry will be discussed in Section III.

### III RESPONSES OF ORGANISMS TO FLUOROACETATES

The majority of studies of a pharmacodynamic character have been made with methyl or sodium fluoroacetate. Some work has been done with fluoroethanol, but little beyond the determination of toxicity has been done with most of the other two-carbon analogs. There has not been a comparable amount of work with the four- and six-carbon compounds and it is possible, although not probable, that some modification of the conclusions concerning these compounds may be necessary in the future.

#### A Absorption

The fluoroacetate compounds are all absorbed to some extent from all sites of application, although the lower members of the series are irregularly absorbed through the skin. Thus, application of methyl fluoroacetate in doses of 100 mgm/kgm to the plucked skin of guinea pigs causes no poisoning according to Foss (52), although Saunders and Stacey (110) report an  $\text{LD}_{50}$  of 20 mgm/kgm for methyl fluoroacetate placed on the clipped back of rabbits. The longer chain compounds and higher esters appear to be more readily absorbed, for example, 2-ethyl hexyl fluoroacetate on the shaved ear of a rabbit is lethal in 6 to 10 hours at a dose level of 10 mgm/kgm (65). It is apparent that these compounds are not readily absorbed through the unbroken skin, but caution should be observed, especially when handling compounds likely to be oil-soluble.

Absorption through the pulmonary epithelium is very efficient in the case of the members of this series studied so far, and absorption of dusts of sodium fluo-

roacetate is equally effective (110) It is sufficient to point out that such data as are available indicate the same degree of toxicity for these compounds when they are inhaled as the esters as when they are injected as sodium salts For example, Saunders and Stacey (110) have reported an  $LC_{50}$  in rabbits for methyl fluoroacetate of 0.1 mgm /l (10-minute exposure) This corresponds roughly to their intravenous  $LD_{50}$  of 0.25 mgm /kgm

There is no noteworthy difference between the toxicities of orally, subcutaneously, intramuscularly, intraperitoneally or intravenously administered methyl or sodium fluoroacetate (30, 93, 129) Very slight differences observed in the unexpected direction of an increased toxicity after oral administration (30) are probably not significant Buckle, Pattison and Saunders (25) caution that methyl fluoroacetate is less toxic when injected subcutaneously in propylene glycol than in sodium chloride solution Because of (1) solubility, (2) stability and (3) the long latent period before symptoms can be produced, which allows time for absorption, it may be concluded that there is no important difference between any non-percutaneous route of administration This is an uncommon occurrence in pharmacology

### *B Distribution in Body Tissues*

Knowledge of the distribution of fluoroacetate is largely inferential in origin It might be assumed that the readily water-soluble sodium or methyl fluoroacetate would be distributed fairly evenly throughout body water Recently, Ramsey and Clifford using their method for the determination of fluoroacetate, have presented data on orally poisoned rats (7 mgm /kgm) which indicate an even distribution of fluoroacetate between the brain, heart, liver and kidney Now that a method capable of detecting small amounts of fluoroacetate in animal tissues is available, it would be extremely desirable to ascertain whether the distribution of fluoroacetate is different in those species in which the heart or central nervous system is primarily affected In this connection one experiment might be cited Two dogs of approximately equal weight ate equal portions of heart muscle or skeletal muscle from a poisoned horse and were apparently capable of differentiating the amount of available fluoroacetate inasmuch as the dog eating the heart muscle died (53) It is common for domestic animals eating poisoned rats to be killed by the fluoroacetate still present in the rat (114)

### *C. Detoxication and Excretion*

Observations that animals may be killed by the fluoroacetate remaining unaltered in poisoned animals or excreted unchanged in the urine (106) suggest that fluoroacetate is not changed in the body to any important extent Urinary excretion is the only route so far demonstrated for the removal of the poison

Although cumulation does occur (52, 105), it is not an outstanding characteristic of this poison Administration of one half to one fifth of an  $LD_{100}$  daily will usually result in acute symptoms in 3 to 10 days, but if 3 days elapse between doses, repeated administration can be carried on indefinitely (52, 99) This suggests that elimination of that portion of the administered dose which was actually

exerting a toxic action is accomplished in about 24 hours. One may infer that at least some enzyme-fluoroacetate combinations are reversible.

#### *D Development of Tolerance*

Tolerance to increasing doses of fluoroacetate has been demonstrated in the mouse and rat (35, 105, 127) and possibly exists in the rhesus monkey (37), but it could not be produced in the dog or rabbit (35, 37). Like cumulation, the development of tolerance is not a characteristic feature of the drug's action. The phenomenon is interesting, however, not only in its species specificity but also in its temporal and quantitative aspects. Rats receiving 0.5 mgm/kgm ( $LD_{10}$ ) by any route become largely resistant to the effects of 5.0 mgm/kgm ( $LD_{75}$  for this strain) within more than 4 hours and less than 24 hours, this resistance lasts about 48 hours. It extends only partially to slightly higher challenging doses, and the ratio of doses cannot be extended, animals surviving 5 mgm/kgm (with symptoms) are as sensitive as controls to 15 mgm/kgm on the following day.

During the period of protection after 0.5 mgm/kgm, there is an increase in the ability of the rat to acetylate p-aminobenzoic acid. This may reflect partial inhibition of acetate turnover, resulting in the accumulation of a larger amount of acetate available for acetylation of foreign amines. Such an accumulation of acetate may also exert a protective action against a subsequent and larger dose of fluoroacetate (see sections III, IV).

#### *E Latent Period*

All students of the actions of fluoroacetate have been impressed with the unusually long and variable latent period between the administration of the drug and the development of the characteristic response. This latent period occurs in all species so far studied, following intravenous injection. Application of concentrations of the methyl ester or the salts of fluoroacetic acid, equivalent to those existing in an intact animal poisoned with an  $LD_{50}$ , to the isolated heart and gut of the rabbit, the exposed brain of dogs, rabbits and monkey, or *in vitro* to enzyme preparations usually produces no immediate changes in the behavior of the organ or system. There is ordinarily no difference between these two agents except in the case of the isolated frog nerve preparation which Boyarski, Postel, Rosenblatt and Gerard (18) have found utterly refractory to the sodium salt but sensitive to the methyl ester. Although isolated intact frog muscle is more sensitive to the ester, the sodium salt is not inactive on this preparation (39).

To illustrate the latent period, data taken from current research in this laboratory may be used. An  $LD_{50}$  of sodium fluoroacetate (0.5 mgm/kgm) injected intravenously in white rabbits requires 125 minutes ( $S.E. \pm 12.7$ ) to cause ventricular fibrillation and death. Symptoms of poisoning are not detectable for at least one-half hour after administration of the fluoroacetate. Increasing the dose to 25 or 250 mgm/kgm will shorten the latent period to 20 minutes but cannot provoke the immediate responses characteristic of many drugs. Doses of the order of an  $LD_{50}$  may cause symptoms of poisoning and death of an occasional animal 48 hours or longer after administration.

The four-carbon compounds,  $\gamma$ -fluorocrotonate or the fluoroethyl ester of fluoroacetic acid, are more toxic than fluoroacetate and have a distinctly shorter latent period. For example, intravenous injection of an  $LD_{90-100}$  of sodium fluorocrotonate (0.3 mgm/kgm) kills rabbits in about 60 minutes. Incomplete data indicate that dogs are very little more sensitive to fluorocrotonate in terms of the dose requirement but that the latent period for an  $LD_{100}$  (0.1 mgm/kgm) is markedly shorter (approximately 90 minutes) than the 273 (S.E.  $\pm$  73) minutes for a corresponding  $LD_{100}$  of fluoroacetate (0.1 mgm/kgm). Experiences of this nature suggest that the latent period can be shortened by increasing the chain length, thereby increasing the lipid solubility as well as decreasing dissociation, and thus facilitating cell penetration. No information is yet available on the latent period associated with the long chain compounds studied by Saunders (25, 109), although they are much more toxic than fluoroacetate. It has been shown (15) that the effectiveness of  $2 \times 10^{-6}$  M sodium fluoroacetate in decreasing the oxygen consumption of yeast is greatly increased by a low pH of the medium, a condition which increases the number of undissociated fluoroacetic acid molecules. This has also been found to be true of iodoacetic acid (3). In fact, undissociated molecules of acids are generally credited with being the form which actually penetrates cells (63).

The latent period in rabbits between the administration of sodium fluoroacetate and the onset of ventricular fibrillation or convulsions can be appreciably shortened by the prior administration of large amounts (approximately 1 gram/kgm) of sodium bicarbonate, fumarate or chloride, but it cannot be eliminated entirely (36). The recent work of Hyde, Beckett and Gellhorn (71) has shown that certain agents facilitating cholinergic transmission potentiate many convulsant drugs. In mice, small asymptomatic doses of neostigmine (0.25 mgm/kgm) greatly shorten the latent period of fluoroacetate (20 mgm/kgm) induced convulsions, but do not eliminate it entirely. The decrease is to about one fifth of the control period (37). Neostigmine produced no change in the latent period when administered with fluoroacetate to dogs or rabbits. There does not seem to be any satisfactory explanation of these observations at present.

The latent period associated with fluoroacetates can probably be considered the result of at least two major factors: (1) the ability of the various fluorinated compounds to penetrate the cell, and (2) the time required for disruption of intracellular processes to become manifest as gross organ dysfunction, in principle this second factor is very similar to that which accounts for the latent period of insulin-induced convulsions. The future will decide which of the two factors is the more important.

#### *F Effect upon Intact Animals (30, 52, 53, 93, 105, 129)*

The directly observable effects of an injection of sodium or methyl fluoroacetate, or fluoroethanol, in unanesthetized animals differ in many respects depending upon the species of animal employed (see Table II). They have been most frequently observed in rabbits, dogs, monkeys and rats, and these species seem to encompass the major variations in types of response.

a) **RABBIT (*Cardiac*)** After an intravenous injection of sodium fluoroacetate (0.5 mgm /kgm) in white rabbits, no change in the animal is discernible for about one-half hour. The first effect noted is usually a weakness of the neck and front legs and a decrease in activity. This state may progress to a marked extent but usually remains moderate until the occurrence of a sudden, violent convulsion of a clonic nature, typically associated with a cry. Opisthotonus, mydriasis and blanching of the retina rapidly develop, followed by progressive relaxation, a few gasping respirations and death. If the thorax be opened immediately, the auricles are found to be beating and the ventricles usually fibrillating. Occasional repeated convulsive bouts are always found to be the result of cardiac syncope, when the animals are carefully followed electrocardiographically (30). Fluorobutyrate causes considerable peristalsis and defecation in rabbits, a response not notable with the fluoroacetates. Except for the greater speed of action, fluorocrotonate is qualitatively indistinguishable from fluoroacetate.

b) **Dogs (*Central nervous system*)** The onset of fluoroacetate-induced effects (usually 4 to 5 hours after 0.1 mgm /kgm) in the dog is heralded by a few minutes of barking and howling, "absence" (non-recognition of human presence), actions suggestive of fearful hallucinations, hyperactivity and finally a tonic spasm followed quickly by running movements. Tonic spasms and running movements may alternate or even completely cease, and the dog may appear normal at times, but ultimately the repeated anoxic assaults on the respiratory center during convulsions result in respiratory paralysis. The heart is often markedly slowed during convulsive seizures but rarely ceases activity until some time after the respiration has ceased. Death is typically the result of the effects of repeated and prolonged convulsions on the respiratory center, and never primarily cardiac in origin.

c) **MAN AND RHESUS MONKEY (*Mixed response*)** Although cats and pigs are perhaps more typical of the "mixed response" type of species (see 30), the rhesus monkey is of greater interest because such data as have been accumulated by the reviewer (through the most diverse channels, see also (52)) indicate that the response of adult man to fluoroacetate may be identical with that of the rhesus monkey. Children appear to be more prone to myocardial failure than to ventricular fibrillation as the terminal event, but, in general, they are very similar to the rhesus monkey in their responses to the poison.

As in man, in whom it may prove a diagnostic problem, the convulsive seizures due to fluoroacetate poisoning in the rhesus monkey are strikingly epileptiform. One or two hours after administration of the poison the animal may vomit and becomes apprehensive and seclusive ("The first indication of poisoning in man is the onset of epileptiform convulsions after an initial period of nausea and mental apprehension" (52)). A few minutes later, actions suggestive of auditory hallucinations are followed immediately by nystagmus. Twitching of the facial muscles, often unilateral, heralds the onset of the convulsive seizure. It quickly spreads to involve the pinnae and the masseter muscles. Spread of the convulsive activity over the rest of the body is then very rapid, ending in a jerking, symmetrical convulsion in which the spasmodic, violent jerks may occur at a rate of



3 per second Tonic components are seen but do not dominate the pattern as they do in the dog The animal is apparently unconscious during this period, but, as the seizure passes off, it will gradually attempt to regain its feet and ultimately does so about 30 minutes after the onset of the attack The monkeys appear depressed for some time but often recover entirely from the convulsion A complete second seizure is infrequently seen Generally, the animal becomes weaker over the period of the next few hours (see cardiac status), but is often standing or otherwise exerting himself when suddenly stricken by ventricular fibrillation and death Spontaneous recovery from ventricular fibrillation in the monkey is uncommon

d) *RATS (Depression)* Although convulsions of a tonic nature, preceded by one- or two-hour period of decreasing activity associated with hypersensitivity to external stimuli, are the usual result of the injection of 5 mgm /kgm of sodium fluoroacetate in rats of unspecified ancestry, death is the result of respiratory depression which gradually occurs long after convulsive activity has decreased or entirely ceased Very large rats (over 400 grams) occasionally develop ventricular fibrillation, but, as might be expected from the general experience with fibrillation in small animals, this is uncommon Some of the confusion in reports from different laboratories may be explained by a recent observation of the effects of the same dose of fluoroacetate in two strains of albino rats Male Wistar rats received directly from the Wistar Institute convulse only occasionally and die after a period of respiratory depression lasting 5 to 24 hours Comparable male rats of the Sprague-Dawley strain received directly from their stocks uniformly develop convulsions within 1 or 2 hours after injection and continue to convulse in a manner very similar to the dog Death occurs in 4 or 5 hours (37). Although superficially these rats are identical, Anker (4) has demonstrated a very striking metabolic difference which will be discussed later.

Rats which have survived an  $LD_{50}$  of fluoroacetate for 24 hours differ from most species (which are usually completely recovered in this time if they are to survive at all) in that they are still markedly affected Disinclination to move is immediately apparent and is probably caused by a gross intention tremor which appears when the animal is forced to move An extreme bradycardia can be detected by palpation or electrocardiographically (31) Complete recovery, if it is to occur, usually results within 48 to 72 hours after poisoning Thiamine and atropine have no effect on these phenomena, but the tremor is temporarily quite exaggerated by diphenhydramine in doses that do not affect normal rats (37)

e) *Special responses in various species* Ward and Spencer (129) noted emesis as a strikingly characteristic and early symptom of fluoroacetate poisoning in several species of carrion-feeding hawks and owls Judged from the nature of the animals this is not surprising, nor does the profound sweating noted in poisoned horses by Frick and Boebel (53) appear unusual Watt and Breyer (131) record that symptoms of poisoning in cattle which have eaten "Gifblaar" can be delayed by withholding water from the animal, a point of some theoretical and practical interest, and animals which recover are exceedingly thirsty In this connection it is known that frogs allowed to imbibe water through their skin after

poisoning, which they do to the extent of a 50% weight gain, die more quickly than those kept dry (99). Mice are often anuric despite large amounts of parenteral fluids associated with therapeutic measures (70).

### G Effect on Specific Systems

a) *Cardiovascular* The actions of fluoroacetate upon the cardiovascular system have been studied most extensively by Chenoweth and Gilman (39) who published a number of plates illustrating some of the phenomena described below. In general, most if not all the changes in the circulatory system produced by fluoroacetate can be explained by the action of the poison upon the heart itself, extracardiac effects, if present, are masked by the magnitude of the cardiac events. There is frequently a slight and transitory rise in mean arterial pressure which does not long persist. It is blocked by atropine and has been related by Foss (52) to the nicotine-like action of large doses of fluoroacetate. However, the general pattern is one of declining blood pressure (31, 105). Failure of the myocardial contractile power is steady and has been demonstrated in isolated heart-lung, perfused heart and papillary muscle preparations (31) as well as by direct inspection (105).

Constriction of the coronary arteries does not appear to occur and is certainly not the prime cause of the cardiac irregularities. During the course of poisoning no changes in capillary permeability to protein-bound dyes or in hemoconcentration, as indicated by hematocrit readings, have been observed (31). An elevation of hemoglobin levels in the goat has been ascribed to splenic contraction (52). As the heart decreases in contractile power it loses the ability to elevate the blood pressure in response to epinephrine or to compression of the descending aorta.

The development of numerous arrhythmias is apparent even on routine kymography, but the bizarre changes which occur can only be appreciated by electrocardiography, preferably by some technic permitting continuous visualization of the rapidly fluctuating changes. There appear to be differences even among cardiac-sensitive species in regard to the types of changes noted in the heart. Common to all, however, is a notable elevation in the amplitude of the T wave, although this perhaps most marked in the monkeys. Progressive downward shifting of the pacemaker was seen in the horse, goat (31) and sheep (105) and the electrical signs of activity in the auricle often disappeared. Prolongation of the P-R interval in the progressive fashion described by Wenckebach was especially marked in the goat, but was also seen in the cat. In direct opposition to this, the rhesus and spider monkeys showed no changes in auricular activity nor in auriculo-ventricular conduction.

Ventricular premature contractions are seen in nearly all species and are especially prominent in the rabbit and monkeys where they occur at first in a peculiarly systematic fashion (1 2, 1 4, etc.). Shifting alternation of the cycle length, QRS voltage, T wave height, shape, direction or take-off is very marked in the monkey and is often not predictably related to a *pulsus alternans*. The alternation of the pulse extends ultimately to a uniform 50% pulse deficit. This has also been reported in at least one fatal human poisoning. Ventricular fibril-

lation may occur at any time, but usually when the heterotopic ventricular arrhythmias are prominent, it appears to be initiated by mechanisms similar to those described by Wiggers for electrically-induced ventricular fibrillation. The occurrence of auricular fibrillation has not been noted in any species.

The actions of fluoroacetate upon the sensitive mammalian hearts are directed toward decreasing contractile power and disorganizing conduction and excitation. Failure or fibrillation as the end result is a manifestation of the relative importance of these effects in different species. It is not known that these effects are all the result of the same action of fluoroacetate, but it may be recalled that the specialized conduction tissue of the heart is contractile muscle tissue as well, suggesting that only a single action may be involved. There appears to be no relation between the gross amount of Purkinje tissue in the various species (56) and the development of conduction defects.

b) *Nervous system* The magnitude of the direct effects of fluoroacetate upon the nervous system of sensitive species is apparent upon inspection of a poisoned animal. It must be emphasized that anoxic convulsions arising from cardiac syncope, such as ventricular fibrillation (in the rabbit, for instance), should not be confused with convulsions resulting from a *per primum* action of fluoroacetate upon the central nervous system, such as occurs in the dog. As indicated in Table II, both types of convulsions may occur in some species. This section will deal exclusively with seizures arising from a direct effect of fluoroacetate upon the nervous tissue. The pattern of these convulsions in intact animals has been described in section III F. With the exception of the remarkable similarity of fluoroacetate-induced convulsions in rhesus monkeys and in man to a *grand mal* epileptic seizure, the gross character of the convulsions in other species is not of great interest.

The regions of the central nervous system affected by fluoroacetate do not appear to be very sharply circumscribed. Studies on peripheral nerve have been carried out for the purpose of elucidating the mechanism of action of fluoroacetate, and will be discussed in section IV. The spinal cord can be shown to be sensitive (a) by local application of fluoroacetate to the cord (34), with convulsive activity developing in a discrete area, (b) by the occurrence of convulsions below the level of a cord transection following an intravenous injection (34), and (c) by experiments in spinal and decerebrate cats (52). It usually requires supralethal doses to demonstrate involvement of the cord and it is probably of no importance in the intact animal. Curiously, spinal rhesus (?) monkeys twitch after 100 mgm/kgm of methyl fluoroacetate but do not convulse (52). Reflexes mediated through the spinal cord of the cat are accentuated in the first few minutes of fluoroacetate-induced activity, but convulsions soon intervene and make further study impossible (52).

Recordings of the electrical activity of the brain, either directly from the brain surface or from the calvarium, of curarized or anesthetized animals have been made by Ward (126) and by Chenoweth and St. John (34). Using cats, Ward found that large doses of sodium fluoroacetate (20 mgm/kgm, 10 times the  $LD_{50}$ ) injected intravenously or into the lateral ventricles produced marked

increases in electrical activity of subcortical areas, particularly the thalamus and hypothalamus. A high frequency, rather low amplitude activity was found to be particularly characteristic of the thalamus, while bursts of slow waves of a 3 to 8/sec frequency in the cortex were noted which were synchronous with the envelope of the fast spikes of the thalamus. Slow wave activity of the hypothalamus was not regularly reflected in the cortex.

When smaller intravenous doses of fluoroacetate ( $\frac{1}{2}$  to 2 times the  $LD_{100}$ ) were administered to dogs under similar conditions, Chenoweth and St John found an increased frequency and amplitude of waves recorded from temporo-parietal and occipital regions of the cortex but relatively little change in activity of frontal areas or cerebellum. Local application of fluoroacetate, either as the sodium salt or the methyl ester, resulted in relatively local changes in activity which spread so slowly as to suggest the probability that diffusion of the poison rather than primary radiation of the electrical activity was the cause of the increased involvement. They have stressed the apparent similarity of the spike and dome pattern often seen during the action of fluoroacetate to the electroencephalographic pattern of clinical *petit mal* epilepsy.

Electrical activity of the cortex reaches very high potentials during fluoroacetate poisoning but it can be obliterated by barbiturates and anticonvulsants (34) as well as by narcotic concentrations (9) of carbon dioxide (126). The sensitivity of rats to electrically-induced convulsions is increased about ten times by fluoroacetate (52). The apparent potentiation by neostigmine has been mentioned.

Rabbits poisoned by intravenous injections of fluoroacetate never reveal any electroencephalographic abnormalities until ventricular fibrillation occurs, but their brain tissue is not entirely resistant to fluoroacetate for convulsions typical for the dog can be induced in rabbits by the administration of sodium or methyl fluoroacetate directly into the cerebrum. This is, in a sense, a corollary to the observation that dogs prepared for electroencephalographic recordings under curare and artificial respiration occasionally develop ventricular fibrillation many hours after large doses of fluoroacetate, a fact indicating that the dog heart is not completely resistant to fluoroacetate (34).

c) *Other systems* Because death from acute fluoroacetate poisoning is the result of cardiac or respiratory arrest in a relatively short and unpredictable period, there is little opportunity to observe changes in systems other than the heart or nervous system. The actions of fluoroacetate are probably exerted upon nearly all actively metabolizing tissues of the body, but the effects are difficult to demonstrate *in vivo*.

Skeletal muscle may be directly affected, notably in rabbits in which there is early head-drop and fore-limb weakness, these effects do not seem to be primarily the result of the lowered blood pressure, although the point has not been proven. Frog skeletal muscle is affected by fluoroacetate *in vitro* (38, 39). Contractions of isolated rabbit intestine are depressed by concentrations of sodium fluoroacetate of the magnitude presumed to exist in animals poisoned with an  $LD_{100}$  (50, 132).

Although kidney tissue is obviously very sensitive *in vitro* (see Section IV), renal dynamics do not seem to be much affected *in vivo*. Himwich *et al* (62) noted an insignificant decrease in glucose Tm in chronically poisoned dogs, but cumulation of the poison occurred and convulsive death precluded the development of serious kidney malfunction. In 5 chronically poisoned cats (0.1 mgm / kgm per day), with deaths at 3, 8, and 10 days and two survivors, high blood non-protein nitrogen levels of 134 and 120 mgm % were noted in two cats at 8 and 10 days, respectively, this suggests definite renal damage (99).

Overall hepatic status is difficult to assess under any circumstances. A currently popular test of at least one function is the duration of anesthesia induced by short-acting barbiturates in laboratory animals. It has become apparent in recent studies in this laboratory that in fluoroacetate-poisoned animals there is a very great prolongation of the anesthetic effects of sodium thiopental, sodium pentobarbital, sodium phenobarbital and possibly even sodium barbital. The effect is not permanent, and the return to a normal duration of anesthesia is complete in less than a week. This has been observed in mice, rabbits, dogs and a rhesus monkey. It appears likely that it is the result of failure of the fluoroacetate-inhibited liver to detoxify these barbiturates, since the response to them is prolonged in proportion to the degree to which hepatic detoxication is believed to be important in their elimination. Studies on this and several related phenomena are currently in progress.

### H Pathological Changes

a) *Anatomical* The histopathologic changes in fluoroacetate poisoning have not been described at length, perhaps because they do not contribute much to an understanding of its action. They appear to be largely the result of progressive cardiac failure with congestion of the abdominal viscera and lungs (52, 105). In the chicken there may be generalized petechial hemorrhages, especially noteworthy in lungs and ovaries (42), but this phenomenon appears quite specific for this species. Ordinary pathological studies do not seem to have been especially helpful in this field, nor are they likely to be so in the future.

b) *Biochemical* Changes in the blood and tissue levels of various metabolites have been studied in a number of species of animals poisoned with fluoroacetate. A consistent increase in blood glucose levels, occasionally to 400 mgm / 100 cc, has been reported in rabbits (94) and goats (52). Excretion of glucose in the urine may be expected to follow such a rise in blood sugar and does commonly occur in poisoned animals. Possibly some of the extra glucose in the blood is derived from liver glycogen since it has been found that poisoned rabbits have a marked reduction in liver glycogen (94). Lactic acid blood levels are elevated in poisoned rabbits (94, 95, 99), but it is exceedingly difficult to define the exact significance of such a change. Pyruvic acid blood levels rise and the lactate:pyruvate ratio is also increased (94). Preliminary experiments have indicated recently that blood and urine levels of acetic acid are elevated in dogs (37).

A considerable and rapid rise in serum inorganic phosphate has been reported in goats (52) and rabbits (95). Some of this phosphate may come from muscle

because rabbit heart muscle shows a marked decrease in total acid-soluble phosphorus and organic phosphorus (95) Plasma levels of other important electrolytes are found to be elevated, particularly just before death occurs Foss has reported changes in plasma potassium from control levels of 17 mgm /100 cc to 25 mgm /100 cc after poisoning has progressed (52) Others report minor increases in potassium and calcium (95) These electrolyte changes are probably nonspecific and reflect the morbidity of cells weakened by an attack at some other point, similar electrolyte alterations occur under many conditions

#### IV THE INTIMATE MECHANISM OF ACTION

A great deal of illumination has been cast upon the manner in which a dose of a fluoroacetate compound brings about the death of an animal Having been assured that the heart or central nervous system is ultimately disabled by events familiar to pharmacologists, one may properly inquire, "How do these events come about"? Indeed, this is currently the most interesting aspect of the fluoroacetate problem

There is no longer any reason to believe that fluoroacetate is in any way pharmacologically analogous to iodoacetate, although this was a natural *a priori* assumption The behavior of poisoned animals, the chemical character unrelated to iodoacetate and the failure of low concentrations to inhibit significantly any system sensitive to iodoacetate seem sufficient reasons to discard this concept entirely In addition, even though the chemical nature of fluoroacetate suggests no basis for the assumption that fluoride ion might be split off and be the cause of the toxic action of fluoroacetate, the actions of the fluoride ion are entirely different from those of fluoroacetate One must of necessity conclude that the fluoroacetate ion exerts its action as such and not because of any obvious chemical reactions with it or because of toxic breakdown products

British workers have succinctly summarized their earlier efforts to identify the systems attacked by fluoroacetate as follows "No enzyme system has been found which is inhibited to any extent by methyl fluoroacetate" (96) In a most important communication in 1947 describing work carried on in 1944, Bartlett and Barron (12) have reported experiments on the metabolism of animal tissues from which they conclude that, "Fluoroacetate probably acts by inhibiting the formation of "active" acetate (the so-called  $C_2$  compound, which may be an acetyl derivative or an acetate radical)" Because of the importance of this provocative conclusion, it is appropriate to review the observations upon which it is based as well as some experiments which do not justify this conclusion

##### *A Tissue Slices, Homogenates and Microorganisms*

It was found that 0.001 to 0.2 M fluoroacetate decreases the oxygen uptake of tissues Tissue slices from rats moribund after an  $LD_{50}$  of fluoroacetate were found to oxidize acetate 20 to 30% less vigorously than control slices The degree of inhibition of acetate oxidation could be increased by adding fluoroacetate to normal tissues *in vitro*, inhibitions as great as 90% occurred in heart slices after 0.005 M fluoroacetate was added This inhibition might be interpreted as

occurring *pari passu* with cellular morbidity except for the fact that the inhibitions of  $O_2$  uptake of guinea pig brain (which does oxidize acetate and does convulse) and of rabbit brain (which does not oxidize acetate and does not ordinarily convulse) were found to be 53% and 11%, respectively

Experiments were then performed to ascertain the mechanism of acetate blockade and several known routes of pyruvate and acetate metabolism were tested (1) *Pyruvate*  $\rightarrow$  *acetate* The oxygen consumption of kidney slices in the presence or absence of pyruvate was decreased to about the same extent (60%) by 0.01 M fluoroacetate, yet in the presence of pyruvate, which apparently was oxidized, acetate accumulated. It is apparent that the further oxidation of this acetate had been inhibited (2) *Acetoacetate*  $\rightleftharpoons$  *acetate* The utilization of acetoacetate by rat kidney slices was completely inhibited by 0.02 M fluoroacetate. Conversely, the formation of acetoacetate from acetate was accelerated by fluoroacetate and this was still further increased as the proportion of oxygen in the gas phase was increased, reaching 233% of normal in pure oxygen. One may infer from this that acetoacetate is converted *in toto* to acetate, the further oxidation of which is inhibited so that acetate accumulates as the end-product of the reactions  $\text{glucose} \rightarrow \text{pyruvate} \rightarrow \text{acetate}$  and forces the reaction  $\text{acetate} \rightarrow \text{acetoacetate}$  (3) *Pyruvate*  $\rightarrow$  *acetate*  $\rightarrow$  *succinate* The formation of succinate from pyruvate was 77% inhibited by 0.02 M fluoroacetate. Since none of the individual reactions of the Krebs' tricarboxylic acid cycle was found to be affected by fluoroacetate (see also (84) for a statement of disagreement), this may be interpreted to indicate that pyruvate enters the Krebs' cycle in large part through acetate and not directly. (4) *Other reactions* Pyruvate and lactate formed by deamination of alanine accumulate in rat kidney slices in the presence of fluoroacetate, a fact that might be readily predicted if it were established that oxidation of pyruvate through acetate is inhibited. Glucose metabolism is directed toward lactate (aerobic glycolysis) to the extent of nearly one half, but the anaerobic utilization of glucose is completely unaffected in kidney slices. Aerobic oxidative synthesis of carbohydrate from pyruvate and acetate is markedly inhibited by fluoroacetate, this fact suggests that, as the entry of acetate into Krebs' cycle is inhibited, this cycle may be a pathway for the synthesis of carbohydrate from pyruvate and acetate. Anaerobic conversion of pyruvate to lactate and acetate was not affected (see also (26)). (5) *Acetylation of foreign amines* The acetylation of sulfanilamide and of p-aminobenzoic acid by rabbit liver slices was increased by 0.02 M fluoroacetate. (This has been confirmed *in vivo* in rats and rabbits (37, 123).) The chemical reaction of acetylation is not affected, formation of acetylcholine from choline in the presence of glucose or pyruvate is not affected by 0.02 M fluoroacetate (see also (85)). Therefore, it may be assumed that the increased acetylation of foreign amines is the result of an inhibition of acetate metabolism in consequence of which more acetate becomes available for acetylations.

Having been thus provided with a basis for the concept that fluoroacetate interferes with the oxidation of acetate in various animal tissues, Kalnitsky and Barron (77) studied the details of the phenomenon in baker's yeast and bacteria

The oxidation of acetate by baker's yeast was 95% inhibited by 0.001 M fluoroacetate (30% by 0.00001 M), only 5% by 0.001 M bromoacetate and not at all by 0.001 M chloro- and iodoacetate, the specificity of the reactions involved are thus demonstrated. Fluorobutyrate and fluorocrotonate (0.001 and 0.003 M) had no inhibitory action at all. The oxygen uptake of yeast suspensions was nearly completely inhibited by fluoroacetate added 15 minutes before acetate, and practically unaffected when the two were added together. Appreciable reversal of such fluoroacetate-induced inhibition could be obtained by adding higher concentrations of acetate (0.1 M). Additional evidence for the specificity of the inhibition was obtained when it was found that acetate oxidation of specially washed yeast was completely inhibited by 0.00075 M fluoroacetate while pyruvate oxidation was only 79% inhibited.

By the use of a different approach, it was reasoned that if fluoroacetate is a specific inhibitor of acetate oxidation there should be no immediate inhibition of the  $O_2$  uptake associated with the oxidation of ethanol to acetate through acetaldehyde. This was found to be the case, oxidation of ethanol by baker's yeast in the presence of 0.01 M fluoroacetate progressed exactly as in the absence of fluoroacetate until the accumulation of unoxidized acetate affected the rate of oxidation of ethanol to acetate. Less complete inhibition of acetate oxidation produces less block of ethanol oxidation. Black and Hutchens (15) found that 0.001 M fluoroacetate did not completely prevent the continuation of ethanol oxidation through acetate.

The anaerobic dissimilation of pyruvate to acetate and formate by *Escherichia coli* was unaffected by 0.01 M fluoroacetate although the oxidation of pyruvate to acetate by this organism was definitely inhibited. *Neisseria gonorrhoeae* does not dissimilate pyruvate but does oxidize it directly to acetate. This reaction is 40% inhibited by 0.01 M fluoroacetate, 26% by 0.02 M acetate and 52% by both together. These experiments add emphasis to the view that inhibition of pyruvate oxidation by fluoroacetate is due to the accumulation of acetate, since even acetate alone is slightly inhibitory.

Quite different results were obtained when another microorganism, *Corynebacterium creatinovorans*, was studied. An increase in the endogenous respiration of this organism was produced by both fluoroacetate and fluorobutyrate. It was suggested that this is the result of diversion of cellular metabolism by fluoroacetate toward oxidative pathways in a manner similar to that produced by low concentrations of cyanide and azide. Although acetate oxidation by this organism was completely inhibited by fluoroacetate, fluorobutyrate had no effect at all.

As was mentioned, the greatest inhibition of acetate oxidations (as measured by decreased  $O_2$  uptake) by fluoroacetate occurred when fluoroacetate was added to the yeast some minutes prior to the addition of the acetate substrate. After two hours the inhibition apparently decreased considerably (as measured by increased  $O_2$  uptake), a fact which Kalnitsky and Barron interpreted as the indirect result of the slow accumulation of citrate and its increasing movement into the carboxylic acid cycle. The same characteristic decrease of inhibition was noted



in the growth curves of *Tetrahymena geleii* in a glucose but not in an acetate medium (48). A further study of the effect of fluoroacetate upon citrate formation was later made by Kalnitsky (75, 76). Using rabbit kidney cortex homogenate, he found that not only fluoroacetate but barium and magnesium salts as well appear to increase the formation of citrate from oxaloacetate. The effect of barium and magnesium is the result of inhibition of citrate utilization. On the other hand, the slight inhibition of citrate utilization (at fumarate? (84), *vi*) produced by high concentrations of fluoroacetate can only account for about 60% of the accumulation of citrate in the presence of fluoroacetate. It was concluded that the increased citrate content might be the result of the inhibition of acetate oxidation reflected through a series of reversible reactions in a manner analogous to the effect of malonate on pyruvate oxidation.

Another approach to the problem of the greater inhibition of fluoroacetate on acetate oxidation by yeast when the fluoroacetate was added before the acetate was made by Black and Hutchens (15). Working in different laboratories, they confirmed the general fact that a more prolonged inhibition of acetate oxidation is obtained by allowing a longer period to elapse between the addition of the inhibitor and the substrate. They suggest that this is similar to the delay noted by Lynen (91) in starved yeast before acetate oxidation becomes vigorous and that it is the result of cellular depletion of certain substances found by Lynen to be essential for acetate oxidation in yeast. Ethanol was found to be particularly efficacious in accelerating the oxidation of acetate by yeast either untreated or pretreated with fluoroacetate.

When calculations of the oxygen consumption of yeast were made using the final rate after equilibrium has been reached, Black and Hutchens found that pyruvate oxidation is more sensitive to fluoroacetate than is acetate oxidation. Because the delay in oxidizing pyruvate is less than for acetate, these workers decided that the earlier conclusion of Kalnitsky and Barron that acetate oxidation is more sensitive than pyruvate was the result of an error in technic. Hutchens, McMahon and Podolsky (69) have recently reported that the inhibition by fluoroacetate salts of pyruvate-induced oxidations in yeast depends on the pH of the medium. However, inhibition of acetate-induced oxidations in yeast and *Chilomonas paramecium* is independent of pH. While this may have been one of the actual causes of differences in opinion concerning the specificity of fluoroacetate-induced inhibitions in yeast metabolism, Hutchens *et al* find pyruvate oxidation much more sensitive than acetate oxidation in the case of *Chilomonas*. Although it is very hazardous to change species in the middle of an argument about fluoroacetate, it can be pointed out in support of this that Bueding (26) proved that in the filarial worm, *Litomosoides carini*, pyruvate oxidation is very much more sensitive than acetate. In addition, he stated, "No evidence has been obtained that fluoroacetate inhibits the respiration of the filariae because of a competitive inhibition of acetate oxidation." Fluoroacetate (0.001-0.004 M), while producing a decrease in the total respiration and motility of the organism, actually produced an accumulation of pyruvate and a decrease in the formation of acetate from glucose. Although in this study he has rigorously tested these

conclusions, Bueding also demonstrated that the metabolic characteristics of *L. carini* are unique in that they differ from those of other helminths and, indeed, from those of most other invertebrates

The anthropocentric may draw more comfort, therefore, from the results of a later study by Kalnitsky and Barron (78) on the effects of fluoroacetate and fluorobutyrate on fatty acid and glucose oxidation in kidney homogenates. Homogenates of rabbit kidney cortex oxidize acetic and many other fatty acids vigorously. The oxidation of acetate was immediately, and practically completely, inhibited by 0.001 M fluoroacetate and fluorobutyrate. This is in contrast to the total lack of effect of fluorobutyrate on acetate metabolism in yeast. This discrepancy was shown *not* to be the result of conversion of fluorobutyrate to fluoroacetate by kidney. Two other sharp differences between yeast and mammalian tissue were noted. The apparent release of the fluoroacetate-induced inhibition of yeast metabolism with time does not occur in kidney suspensions nor does ethanol have the least effect on the degree of acetate oxidation. Fluorobutyrate proved to be a more potent inhibitor of butyrate oxidation than did fluoroacetate, 0.00005 M fluorobutyrate inhibited butyrate oxidation 86% while at the same concentration fluoroacetate produced only 32% inhibition. Oxidation of higher fatty acids was also inhibited to varying extents by both fluoroacids.

In contradistinction to the case in yeast where it developed slowly, glucose oxidation was rapidly inhibited by fluoroacetate in kidney homogenates, as was also that of acetate. However, as in their previous studies, they found that pyruvate oxidation was not inhibited until a considerable portion (20%) of the added pyruvate had been oxidized. It appears that the specificity of fluoroacetate inhibition described for yeast is not to be found in mammalian tissue, in this case, rabbit kidney. It is interesting that fluorobutyrate is a more effective inhibitor of butyrate oxidation than is fluoroacetate. One can only speculate at this time about the effects of appropriately fluorinated higher fatty acids.

By actual analysis for acetate, Colowick, Berger, Slein and Corn (41) found that rabbit kidney cortex homogenates removed about 35% less acetate after addition of 0.005 M fluoroacetate. They add the new information that the extra oxygen consumption caused in dialyzed extracts of rabbit kidney cortex by addition of various metabolites is inhibited by high concentrations of fluoroacetate (0.05 M) to varying degrees. Glucose is most sensitive, the extra oxygen uptake being inhibited 68%, and phospho-enol pyruvate is approximately the same, the inhibition being 65%. Unfortunately, acetate does not appear to have been tested. The effect of fumarate was found to be inhibited 53% but other metabolites and components of the tricarboxylic acid cycle were relatively little affected. The curious sensitivity of fumarate-induced extra oxygen consumption does not appear to be entirely coincidental for Boyarski, Postel, Rosenblatt and Gerard (18) found it to be specifically effective in preventing the decrease in the action potential of methyl fluoroacetate-poisoned frog nerve. On the other hand, it actually increased the toxicity of sodium fluoroacetate to intact rabbits (36). These matters require considerable clarification. Liébecq and Peters (84) have reported recently that studies with centrifuged, homogenized guinea pig kidney

and pigeon brain preparations indicate the possibility that a "fluoro- $C_2$  active fragment" may be formed which enters the tricarboxylic acid cycle and becomes an inhibitor of this cycle. They found, as did Kalnitsky (76), that citrate accumulates during poisoning *in vitro* while acetate does not. The relation of results of many *in vitro* studies to the events occurring *in vivo* is difficult to establish. It is possible that more physiological preparations could prove more useful.

### B Working Muscles

The use of an actively functioning, normally organized preparation such as the frog sartorius muscle has led to some additional and slightly different information. Colowick, Berger, Slein and Cori (41) found that the oxidative resynthesis of phosphocreatine by frog sartorius following one minute of tetanic stimulation was depressed as much as 40% by previously soaking the muscle for 1 hour in 0.005 M methyl fluoroacetate. These findings were explained as the indirect result of depressed tissue respiration, for the simultaneous oxygen uptake of these muscles following stimulation was decreased from an average 150% increase to only 35% increase. Essentially the same results were obtained when dinitrophenol was used to increase basal oxygen consumption, for 0.005 M sodium fluoroacetate inhibited the increase about 70%.

Similar results were obtained when caffeine was used by Clarke and Riker (39) to stimulate the frog sartorius, the excess oxygen consumption of muscles contracting under such circumstances being decreased by methyl fluoroacetate. The respiration of resting muscles is not affected significantly at similar concentrations of methyl fluoroacetate. The oxidative recovery heat which normally follows a single maximal twitch was abolished by 0.001 M methyl fluoroacetate or 0.01 M sodium fluoroacetate, yet the muscles continued to contract, exactly the reverse of the situation with iodoacetate where an increasing oxidative recovery heat production may accompany contractile failure. The depression of the activity oxygen consumption of these muscles induced by fluoroacetate can be abolished completely by 0.01 M acetate, glycerol monoacetate, pyruvate and ovalacetate. Ketoglutarate, fumarate, malate and succinate were less effective, and glucose and lactate were without any significant effect.

In a continuation of this study, Clarke and Riker have recently found that, unlike iodoacetate, fluoroacetate does not inhibit anaerobic glycolysis and, under the conditions of their experiments with frog muscle, the rate of glycolysis is actually increased. Because the aerobic accumulation of lactate that normally results from muscle activity is significantly less in the presence of fluoroacetate and since lactate formation is not inhibited by fluoroacetate, it appears that lactate must be metabolized in the poisoned muscle. The explanation of these results is to be found in the fact that, in this preparation at least, the action of fluoroacetate appears to be to inhibit oxidative carbohydrate breakdown, as a consequence, the anaerobic carbohydrate breakdown which occurs in activity is enhanced, and this results in a rapid turnover of lactate without its accumulation. It is evident that this will provide sufficient energy to permit muscular contraction.

Three independent studies of the effect of fluoroacetate upon the spontaneous contractility of isolated upper segments of rabbit small intestine have been made. The effectiveness of acetate as a source of energy for the contraction of otherwise substrate-free preparations of the type described by Furchgott and Shorr (55) led Furchgott to study the interrelations of fluoroacetate and acetate on this preparation. Experiments performed during 1946 by Furchgott (54) indicated that glucose could supply energy for contraction of intestinal smooth muscle in the presence of fluoroacetate under either aerobic or anaerobic conditions, although acetate could not. Fluoroacetate poisoning was irreversible when carried out under aerobic conditions. However, if fluoroacetate was added during a period of anoxia, allowed to remain in the muscle chamber for over 30 minutes, and then washed out before the restoration of oxygen to the muscle, there was no toxic effect.

Later, Farah, West and Angel (50), upon examining this system found that both glucose and acetate were effective antagonists to fluoroacetate, and that there are considerable differences in the character of the response of the gut to fluoroacetate in the presence of these substrates. They have shown that although fluoroacetate depresses contractility more rapidly when glucose is the sole substrate than when acetate alone is present, the percentile decrease in amplitude of contraction at a given concentration of fluoroacetate is greater in the presence of acetate than when glucose is the substrate. There is a direct relation between (a) the time required for 0.0008 M sodium fluoroacetate to produce 95 to 100% inhibition of contraction and (b) the concentration of sodium acetate present in the bath, the higher the concentration of acetate the longer the time required for this inhibition to result. Butyrate and pyruvate, when they are the sole substrates, are not detectably different from acetate with respect to the percentile reduction in contraction amplitude produced by a given concentration of fluoroacetate.

Although regular contractions in glucose are stopped by high concentrations of fluoroacetate, there remains an irregular, high amplitude "fluoroacetate resistant" contraction when glucose is present with or without other substrates, this phenomenon is seldom or never seen when acetate alone is the substrate. It was noted by all groups which investigated the problem. Farah *et al* (50) have found that only mannose acts like glucose whereas galactose, fructose, pyruvate, butyrate, caproate, caprylate, succinate, fumarate and  $\alpha$ -keto-glutarate are, like acetate, unable to support these contractions. These contractions are not abolished by anaerobiosis ( $N_2$ , cyanide) or by malonate, although azide and iodoacetate are effective in abolishing them. It has been known for some time, as Farah *et al* have pointed out, that intestinal smooth muscle can utilize glucose and mannose as a source of contraction energy during anaerobiosis. They feel that it is possible that energy for the fluoroacetate resistant contractions may be obtained from anaerobic glycolytic pathways.

That glucose is more effective than acetate in maintaining motility of gut segments in the presence of high concentrations of fluoroacetate was confirmed by Weeks and Chenoweth (132). When intestinal strips are allowed to contract

to exhaustion in Krebs-Henseleit solution in the absence of substrate, the normal stimulatory action of added 0.005 M sodium acetate is prevented by addition of 0.00032 M sodium fluoroacetate 5 minutes before the sodium acetate, whereas subsequent addition of 0.005 M glucose is still effective. Although they found that glycerol monoacetate is a very effective antagonist to fluoroacetate *in vivo* and sodium acetate is definitely not, in the isolated intestinal segment preparation sodium acetate is nearly five times as effective as glycerol monoacetate against fluoroacetate. Other experiments demonstrated that glucose-induced contractions, after fluoroacetate inhibition (0.01 M) in acetate, were of the same amplitude (approximately 30% of control values) as the contractions which persisted when the same concentration of fluoroacetate was added to muscles with a glucose substrate. These various observations can probably be explained by assuming, and there appears to be good reason to do so, that intestinal muscle under these circumstances obtains energy for contraction from at least two sources: (1) the breakdown of glucose which can occur anaerobically and (2) reactions of the tricarboxylic acid cycle into which acetate enters. When acetate, as the sole available substrate, is blocked from entry into the cycle by high concentrations of fluoroacetate, contraction ceases; in contrast, low concentrations of fluoroacetate are unable to produce a block in the presence of excess acetate. In the presence of glucose, energy for contraction is still available despite the blockade of acetate produced by fluoroacetate. This blockade may be increased until only the fluoroacetate, anaerobiosis-resistant contractions remain.

### C Working Nervous Tissue

According to Shanes and Brown (117), the preservation of the resting potential of frog nerve depends upon formation of pyruvate by the glycolytic cycle and the subsequent aerobic metabolism of this substrate. Because 0.01 M methyl fluoroacetate interferes with the redevelopment of the resting potential of nerve in oxygen following a period of anoxia, Shanes (116) felt that this was sufficient to suggest an interference with pyruvate metabolism. Conversely, the simultaneous addition of methyl fluoroacetate and sodium pyruvate to nerve in oxygen maintains a higher resting potential than when pyruvate is omitted, a fact which suggests a beneficial effect of added pyruvate not noted with acetate. During poisoning, he found that the threshold of excitability of frog sciatic nerve to condense discharges steadily increased.

A series of studies has recently been published which has revealed several new facts about the action of fluoroacetate (17, 18, 22, 23, 45, 98). The sodium salt of fluoroacetic acid is nearly without action on frog nerve or brain *in vitro* although methyl fluoroacetate has several actions. Thus, the ester decreases the action potential of frog sciatic nerve and reduces conduction velocity by a process of blocking conduction in fibers, the threshold of the larger fibers is raised before that of the smaller fibers at concentrations of 0.005 M. The respiration of such nerves is decreased to 20% of normal. Sodium fumarate added before, or up to 15 minutes after, the methyl fluoroacetate protects against the action

potential changes in a 2:1 molar ratio, but the respiration of the nerve may still be 50% decreased. Succinate is equally effective in a 5:1 molar ratio, but ethanol, acetate, pyruvate,  $\alpha$ -keto-glutarate and glucose are ineffective (18). Although their data have not yet been fully reported, Doty and Gerard (45) have found that methyl fluoroacetate will depress the resting oxygen consumption of frog nerve as described, but that the increased oxygen consumption which occurs on stimulation is not affected. Thus, with 0.001 M methyl fluoroacetate the resting  $Q_{O_2}$  is decreased by 25% whereas the activity increase is unaffected and the action potential is undisturbed. They caution, however, that the presence of respiring, non-conducting elements in a nerve trunk must be considered when interpreting these results.

In Gerard's isolated frog brain preparation, sodium fluoroacetate is ineffective whereas methyl fluoroacetate inhibits respiration 45% at 0.012 M (23). At this level the brain potentials are decreased about 50%. At 0.01 M methyl fluoroacetate there may also be a 50% decrease in cholinesterase activity. On this preparation the beneficial effects of fumarate are again seen, but they are rendered somewhat less specific by observations that fumarate protects to some extent against di-isopropyl fluorophosphate and that under certain circumstances as little as 0.000,001 M sodium fumarate can itself induce bizarre electrical changes in the brain.

The inactivity of sodium fluoroacetate does not appear to be solely the result of inability to penetrate cells, for it is inactive on rat brain or dog nerve homogenates in which cells are disrupted. Malic dehydrogenase of rat brain is sensitive to methyl fluoroacetate (20% inhibited at 0.001 M), but in general dehydrogenases are not much affected by fluoroacetate (98).

#### *D Isolated Perfused Hearts*

By the use of a recirculating system for perfusing isolated hearts through the coronary arteries over long periods with bacteria-free Ringer's solutions (32), it has been found that concentrations of methyl or sodium fluoroacetate comparable to those calculated to exist in rabbits or rhesus monkeys poisoned with an  $LD_{100}$  cause a gradual decline in the amplitude of contraction, occasional alternans and rarely, if ever, fibrillation (28). The relative sensitivity of rabbits and monkeys to fluoroacetate is also manifested in their isolated hearts. Essentially the same degree of cardiac incompetence was produced by methyl fluoroacetate acting over a two-hour period in a concentration of 0.00001 m (0.5 mgm / L) on the isolated rabbit heart as by an intravenous dose of 0.5 mgm /kgm in the intact animal (equivalent to about 0.6 mgm /L of body water). In the case of the monkey heart, 0.001 M fluoroacetate in the perfusion fluid produces effects which correspond roughly to those of an intravenous dose of 5 to 10 mgm /kgm.

The substitution of sodium acetate for the glucose of the perfusate in both rabbit and monkey hearts effectively maintained contraction and exerted an extensive protection against the effect of added fluoroacetate (33). In some instances protection was definite when the molar ratio of acetate to fluoroacetate

approximated unity, but usually a higher ratio was necessary. Pyruvate was somewhat less effective in maintaining contractions and in protecting against fluoroacetate, perhaps only because of toxic impurities (20).

Because the presence of acetate prevented the action of practical concentrations of fluoroacetate, it was impossible to demonstrate any change in the utilization of acetate by the heart in the presence of fluoroacetate. However, when failure was produced by the addition of 0.01 M fluoroacetate to monkey hearts with either glucose or pyruvate as the substrate, there was an acetate accumulation of as much as 3 grams/kgm dry weight/hour over the three-hour exposure period. No other change in the utilization or production of acetate, pyruvate, lactate,  $\alpha$ -keto-glutarate or glucose was detected.

Despite the protection exerted *in vitro*, sodium acetate by itself exerts no protective effect whatever against the toxic action of fluoroacetate in the intact rabbit (36, 37).

#### *E Mechanism Studies in Intact Animals, Antidotes*

Because humans accidentally or wilfully ingest rat poisons, there can be no doubt of the desirability of an effective antidote to fluoroacetate. The studies so far described do not offer much hope that any highly effective treatment of well-established fluoroacetate poisoning will be found. Indeed, most investigators have been content if their results with antidotal therapy contribute something to an understanding of the mechanism of action of the poison.

It has been mentioned that sodium acetate, although it is the most likely candidate, is not an effective antidote or prophylactic in rabbits poisoned with fluoroacetate. However, Tourtelotte and Coon (125) have found that in mice, at least, sodium acetate (2 to 3 gram/kgm) will protect against sodium fluoroacetate. Ethanol, which may be considered simply a source of acetate *in vivo* or, more complexly, a catalyst of the Krebs' cycle, is also effective (1.6 gram/kgm). Ethanol and acetate together are distinctly more than twice as effective as either alone, a fact which suggests a synergistic effect.

Hutchens *et al* (70) have reported more fully on the effectiveness of ethanol alone or with sodium acetate in protecting mice against fluoroacetate. Ethanol is also effective in rabbits, and to a lesser extent in guinea pigs, but not at all in dogs. It was noted that barbiturates were somewhat effective in protecting dogs but not mice. Although there is no doubt that the judicious administration of barbiturates (bearing in mind the prolongation of sleeping time described in III-G, c) will control convulsions induced by fluoroacetate in dogs (52, 70, 125), animals which survived in this laboratory manifested changes characteristic of cortical damage. It would thus appear that, although overt convulsions are prevented, the pathological pattern of fluoroacetate poisoning has been unaffected.

The effectiveness of sodium acetate *in vitro*, despite a generally unfavorable response to it *in vivo*, led to a search for other sources of  $C_2$  moieties. One of the most promising has been glycerol monoacetate which has protected rats, rabbits, dogs and rhesus monkeys against fluoroacetate (36, 37). Equimolar doses of

glycerol monoacetate and ethanol, given to rhesus monkeys after fluoroacetate poisoning had progressed, were strikingly different in effectiveness, ethanol apparently being of no value although its effectiveness in mice and rabbits has been confirmed. Several other such compounds have been found which exert a protective action against either fluoroacetate or fluorocrotonate.

The simultaneous administration of large amounts of insulin and glucose is often effective in dogs and rabbits, although neither substance is effective alone (37). (The forcing of glucose is also protective against anoxic anoxia (21)). Anoxia caused by methemoglobinemia (approximately 70%) produced either by sodium nitrite or p-aminoacetophenone is a very effective antidote or prophylaxis for fluoroacetate poisoning in those species in which it can be induced, *e g*, mice and dogs (37). McNamara (97) has recently found that physiological sodium chloride solution exerts a definite protective effect against fluoroacetate poisoning in rabbits, a fact which may account, in part at least, for the heterogeneous character of some of the substances that appear to exert a moderate protective action. It serves no useful purpose to list in detail all those substances which have proven ineffective, but, in general, salts of fatty acids, anticonvulsants, vitamins and most metabolic intermediates are without effect. Potent antifibrillatory, autonomic and cardiac drugs are generally of no therapeutic value, they may act differently after fluoroacetate (*e g*, 50).

#### V MISCELLANEOUS FLUORINATED COMPOUNDS

Several familiar compounds in which fluorine has replaced hydrogen or chlorine have been prepared and are of some interest here. Although 2,3-difluoro succinic acid (80),  $\text{HOOC}-\text{CHF}-\text{CHF}-\text{COOH}$ , appears to inhibit succinic dehydrogenase completely in very low concentrations, it is of singularly low toxicity, the  $\text{LD}_{50}$  in mice and dogs (salt or dimethyl ester) being above 200 mgm/kgm (37). When fluorine is substituted for chlorine in sesqui-H, a very potent analog of mustard gas, the resulting compound  $\text{F}-\text{CH}_2\text{CH}_2-\text{S}-\text{CH}_2\text{CH}_2-\text{S}-\text{CH}_2\text{CH}_2-\text{F}$ , is nontoxic with neither vesicant nor fluoroacetate-like activity (96). This suggests that the body is unable to rupture the C-S link in this compound to obtain fluoroacetate and adds emphasis to the fact that the vesicant action of the mustards is dependent upon reactive halogens. Other compounds in which fluorine has been substituted for an hydrogen atom, such as di- (2-fluoroethyl) fluorophosphate or triethyl lead fluoroacetate, combine some of the characteristic activity of the parent compound with that of fluoroacetate (109). Mention should be made of the potent antithyroid activity reported by Litzka (87) for

3-fluorotyrosine  $\text{HO}-\overset{\text{F}}{\text{C}_6\text{H}_4}-\text{CH}_2\text{CHNH}_2\text{COOH}$ . This activity may be the cause of the high toxicity ( $\text{LD}_{50}$ , 12.5 mgm/kgm), since 1- or 4-fluorobenzoic acids are virtually nontoxic (79, 86). Boyer, Evans and Phillips (19) were unable to demonstrate an effect on the basal metabolic rate of rats, although they confirmed the toxicity of the compound. These compounds have been included because they give some indication of the way in which fluorine atoms in place of



hydrogen atoms may be used to obtain information concerning biological reactions

## VI DISCUSSION

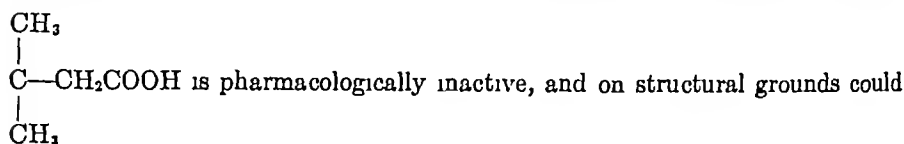
Nearly all the original data on monofluorinated fatty acids so far available in the open literature and much of that in the classified literature have been presented in this review. There appears to be a plethora of information on less important points but a dearth of incontrovertible data on the more important questions. What interpretation can be given the facts now available?

It is clear that the substitution of one fluorine atom for one hydrogen atom of a compound which is known, or suspected, to be of biological importance confers unusual pharmacological properties upon that compound provided that the substitution be made in the proper place. This proper place is always in the terminal position of a straight chain fatty acid containing an even number of carbon atoms, to cite the best-defined series. Thus 2-monofluoroacetic acid is active, 3-monofluoropropionic acid is inactive and 4-monofluorobutyric acid is very active, although 2-monofluorobutyric acid is inactive, and so on. Substitution of any atoms or groups other than one fluorine atom on the terminal carbon atom does not result in characteristic activity. More complex compounds containing a suitable grouping, for example, esters of 2-monofluoroacetic acid or 2-fluoroethanol, exert a typical pharmacological action if they can be broken down in the organism to yield fluoroacetic acid. This principle has been applied in a few less simple cases to indicate whether or not the body can rupture certain linkages, the C-S bonds in the chain  $\text{F}-\text{C}-\text{C}-\text{S}-\text{C}-\text{C}-\text{S}-\text{C}-\text{C}-\text{F}$  being an example. It appears to be a technic of some value, although open to criticism on the grounds that such a compound might be a specific inhibitor *per se*, should it not prove to be inert (for instance, fluoroacetyl salicylic acid).

The toxicity of suitably substituted even-numbered fatty acids in contrast to the inertness of odd-numbered homologs is superficially further evidence for  $\beta$ -oxidation of fatty acids, the assumption being that the even-numbered chains are broken down to the toxic fluoroacetic acid. Then, in order to account for the much greater toxicity (and the relative differences in various species) of the longer chains as compared to fluoroacetic acid on a mole per kilogram basis, it is necessary to assume that (a) they penetrate cells much more efficiently and/or (b) that release of fluoroacetate as an "active"  $\text{F}-\text{C}_2$  radical occurs, preferably directly in the area where it could do the most harm. To the contrary, the quantitative and qualitative differences between the actions of the fluorinated higher fatty acids and the shortest active acid suggest most strongly that they act at separate sites. Evidence has been presented that fluorobutyrate, for example, is a much more active inhibitor of butyrate oxidation in mammalian tissue than is fluoroacetate, while in yeast fluorobutyrate does not inhibit acetate oxidation at all although fluoroacetate actively does so. Under at least one set of circumstances it has been shown that fluorobutyrate does not break down to fluoroacetate. In addition, the gross pharmacological effects of fluorobutyrate differ qualitatively from those of fluoroacetate, although admittedly the difference requires

sharper definition While it is impossible on the basis of available information to exclude the possibility of  $\beta$ -oxidation of fluorinated higher fatty acids to fluoroacetate, it appears probable that they exert their toxic action in part, at least, by interfering with the metabolism of the corresponding non-fluorinated fatty acid in the cell It is thus equally easy to dispose of the inactivity of odd-numbered fluoroacids, for it is generally uncommon to find the non-fluorinated homolog important in the main line of fatty acid oxidation <sup>4</sup>

Certain inactive compounds shown in Table III might be used to add weight to the hypothesis of  $\beta$ -oxidation to fluoroacetate For example,  $F-CH_2-$



not be expected to undergo  $\beta$ -oxidation to fluoroacetic acid Similarly, derivatives of fluorobutyric acid in which carbon atoms 2 and 3 are part of a cyclic structure are inactive, and are not susceptible to  $\beta$ -oxidation Although the inactivity of these compounds adds support to the idea that  $\beta$ -oxidation of higher  $\omega$ -fluoroacids is necessary for activity, it is equally possible to argue that because of steric factors these biologically abnormal compounds never have an opportunity to inhibit butyrate oxidation inasmuch as they are kept from entering a reactive center by their structural deformity and for that reason are pharmacologically inert

In this case the assumption of "entrance into reactive centers" is based entirely upon the remarkable similarity (see Table I) of the  $FCH_2-$  and  $HCH_2-$  radicals, the chief differences being that the fluorine atom is about twice the size of the hydrogen atom and is bound more securely to the carbon atom (Were there no physical-chemical differences at all there would seem to be no reason to expect any pharmacological differences) If it be assumed that in the process of metabolism fatty acids fit some sort of matrix which requires entry of the terminal methyl group, it is easy to visualize the arrival of a fluorinated methyl group which fits effectively, but, perhaps because of the greater hold the carbon and fluorine atoms have upon each other and because of fluorine's propensity for hydrogen bonding, it cannot readily be dislodged In such a case as fluoroacetic acid the prior administration of substances which release large amounts of acetic acid in the cell could be expected to influence this phenomenon by competing for the fixation site Poisoning by fluoroacetate can indeed be prevented or even reversed by such substances in intact animals and in some isolated systems Unfortunately, a different interpretation can be placed upon this observation There is no way of knowing with certainty whether added acetate competes with fluoroacetate or simply by-passes a blockade To clarify this it is necessary

<sup>4</sup> Inactivity may be a relative matter because the doses of odd-numbered compounds tested do not seem to have exceeded 200 mgm./kgm and have been examined only in a few species It is possible that some organisms may be very sensitive if odd-numbered fatty acids are actively metabolized by them

to marshal the data concerning the various possible blockades which have been suggested

Although it would be extremely helpful to have more data on other compounds, nearly all the detailed investigations concerning inhibition of metabolic processes by the fluoroacids have been performed with fluoroacetic or fluorobutyric acid. The specificity (or even the existence) of the inhibition of the oxidation of acetate and pyruvate by fluoroacetate has been the chief subject of study, the results of which have been vastly complicated by differences in technic, species or organs employed. (One searches the literature in vain for mention of the existence of fluoropyruvic acid,  $\text{F}-\text{CH}_2-\text{C}-\text{COOH}$ , which



might be utilized to settle some of these questions )

It is fairly certain that fluoroacetic acid does not inhibit acetylation of foreign or natural amines by mammals. The formation of acetoacetate from acetate is not inhibited and in general—*Luomosoides carini* being a clear exception—the oxidation of pyruvate to acetate may be directly affected while the disposition of the acetate so formed is certainly affected. Acetate probably exerts an inhibitory effect upon pyruvate oxidation after a sufficient amount of it has accumulated. There appears to be no way at this time of choosing between findings which suggest inhibition of fumarate oxidation and those which do not. In this connection it is possible to argue obliquely that, because fluorosuccinate and other potent competitive inhibitors of succinic dehydrogenase are relatively feeble animal poisons, inhibition of other neighboring steps in the Krebs' cycle is not likely to be the cause of death in mammals poisoned with the usual minute doses of fluoroacetate. The effects on citrate and glucose metabolism may best be explained as the indirect result of inhibition at other points.

It is well established that the breakdown of glucose to triose phosphate can occur anaerobically and aerobically, and is a source of much energy. Pyruvate has long been known as the main end-product of this metabolic chain, lactate being a simple conversion product of pyruvate. However, in the last decade the  $\text{C}_2$  fragment, a much discussed entity having many but not all of the characteristics of acetate, has been definitely added as a stage of carbohydrate oxidation beyond pyruvate and as the end-product of fatty acid breakdown (16, 83, 121).

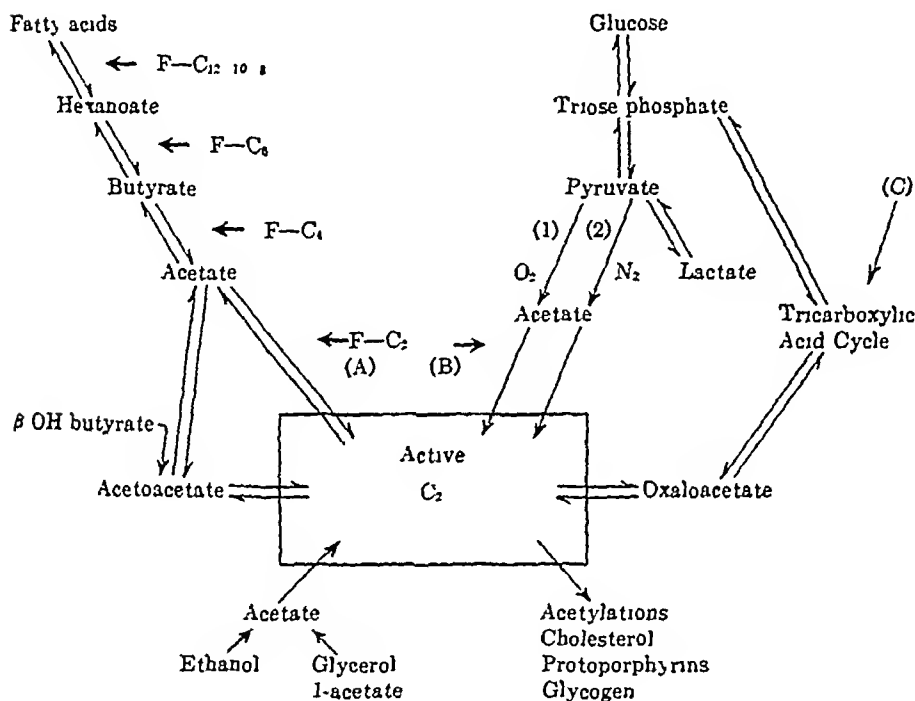
Before presenting the schema depicted in Figure 1, it is necessary to bring out certain less well known observations which relate to the possibility of its general applicability and to the action of fluoroacetate. Differences among species with regard to the action of fluoroacetate must be more than a matter of vagary. If the inhibitions of various metabolic systems and pathways and the responses of intact animals which have been reviewed here are considered in the light of the importance of these systems and pathways to the economy of the organism being studied, some interesting relations are obtained. Thus, although there are no qualitative differences between Wistar and Sprague-Dawley strains of rats in the way they handle the acetate in their acetate pool, Wistar rats turn over less acetate (12 to 15 mM/100 gram/24 hr) than do Sprague-Dawley rats (20

to 25 mM/100 gram/24 hr ) In addition Wistar rats are unable to convert pyruvate to acetate although Sprague-Dawley rats do so freely (4) The difference in response to fluoroacetate is clear, the strain to which acetate is more important is more sensitive to fluoroacetate (although more resistant to pyridoxine deficiency (27) or alloxan (82)) It has recently been shown that intact rabbits metabolize formate actively, but that dogs and man are nearly unable to do so, incidentally, this accounts for the relative toxicity of methanol to these two latter species (88, 89, 90) Dog muscle, on the other hand, can oxidize  $\beta$ -hydroxybutyrate and acetoacetate whereas rabbit muscle can only use acetoacetate (124) The differences between dogs and rabbits in response to fluoroacids are striking Perhaps a similar relation exists when still other species are considered, for isolated mammae of the goat, a fluoroacetate-sensitive animal, utilize acetate whereas those of the relatively resistant rat and still more resistant mouse do not appear to do so (51) Mention has been made of the ability of guinea pig brain to oxidize added acetate and the relative inability of rabbit brain to do so, the fluoroacetate-induced inhibition of acetate oxidation being greater in brain tissue from the guinea pig than from the rabbit The convulsive pattern of fluoroacetate poisoning in the guinea pig and the effects on the heart of the rabbit, which oxidizes acetate vigorously (10, 11, 33), intimate that this is no mere coincidence

When one views these scattered observations on the relation between fluoroacetate action and metabolic pathways in the light of the protective effect of acetate and its donors, it is not unreasonable to suspect that the degree of sensitivity of an animal or its organs to fluoroacetate is an indication of a certain characteristic of its acetate metabolism To establish this suspicion as a fact will require much effort and it is probable that the problem is even more complicated than appears at first Reference has been made to studies with isolated frog muscle, rabbit intestine and intact animals which show that there are fluoroacetate-insensitive metabolic pathways giving rise to sufficient energy to maintain function Evidence has been adduced to indicate that the breakdown of glucose is not sensitive to fluoroacetate and that some such system can be enhanced *in vivo* by partial anoxia (methemoglobinemia) or by accelerating the breakdown with an excess of glucose and insulin It is highly probable that in addition to individual peculiarities of the metabolic systems for handling  $C_1$ - $C_4$  molecules in a given species a second factor, the glycolytic rate, is important in controlling the sensitivity of the organ or organism Few comparative data have been published, although Wu and Chang (134) have recently pointed out that the glycolytic rates of isolated eel, toad, turtle and rat hearts decreased (in that order) with their resistance to anoxia Grossly, the sensitivity to fluoroacetate likewise decreases in the direction of greater glycolytic activity It is also well known that very young mammals are very much more resistant to anoxia than are older ones Nine 24-hour old dogs recently tested in this laboratory were markedly resistant to fluoroacetate Further, the relative sensitivity of various vertebrates to tissue anoxia induced by potassium cyanide (67) is strikingly similar to their order of sensitivity to fluoroacetate Farah (50) has called attention to the similarity

between the sensitivities to fluoroacetate, cyanide and anoxia of various portions of the gut, which follow Alvarez's concept of a metabolic gradient. Although it is surprisingly difficult to find accurate comparative data (64), such adult mammals as have been found very resistant to anoxia (21) are also resistant to fluoroacetate.

FIG 1 Tentative Localization of Fluoroacid Blockades



Reaction (1) Oxidative decarboxylation of pyruvate to acetate

Reaction (2) Anaerobic degradations of pyruvate to acetate and lactate. Enhanced by partial anaerobiosis *in vivo* (methemoglobinemia) and by large amounts of glucose, thus minimizing effect of block (B)

Blockade (A) (B) Competitively antagonized by C<sub>2</sub> donors

Blockade (C) and others not shown. Minor blockades caused by imperfect introduction of F-C-C-R in high concentrations into a matrix designed for other structures, *e.g.*, malic dehydrogenase.

In Figure 1, the anaerobic degradation of pyruvate to acetate by coupled oxidation-reduction reactions ("dismutation") to lactate and acetate, or by fragmentation ("disimilation") to acetate and formate, or by still other processes, is shown as unaffected by fluoroacetate, although oxidation of pyruvate or fatty acids through acetate to active acetate is shown as blocked. If allowance is made for variations among species and strains of organisms, such a blockade can account for many of the pharmacological responses so far described. One

to 25 mM/100 gram/24 hr ) In addition Wistar rats are unable to convert pyruvate to acetate although Sprague-Dawley rats do so freely (4) The difference in response to fluoroacetate is clear, the strain to which acetate is more important is more sensitive to fluoroacetate (although more resistant to pyridoxine deficiency (27) or alloxan (82)) It has recently been shown that intact rabbits metabolize formate actively, but that dogs and man are nearly unable to do so, incidentally, this accounts for the relative toxicity of methanol to these two latter species (88, 89, 90) Dog muscle, on the other hand, can oxidize  $\beta$ -hydroxybutyrate and acetoacetate whereas rabbit muscle can only use acetoacetate (124) The differences between dogs and rabbits in response to fluoroacids are striking Perhaps a similar relation exists when still other species are considered, for isolated mammae of the goat, a fluoroacetate-sensitive animal, utilize acetate whereas those of the relatively resistant rat and still more resistant mouse do not appear to do so (51) Mention has been made of the ability of guinea pig brain to oxidize added acetate and the relative inability of rabbit brain to do so, the fluoroacetate-induced inhibition of acetate oxidation being greater in brain tissue from the guinea pig than from the rabbit The convulsive pattern of fluoroacetate poisoning in the guinea pig and the effects on the heart of the rabbit, which oxidizes acetate vigorously (10, 11, 33), intimate that this is no mere coincidence

When one views these scattered observations on the relation between fluoroacetate action and metabolic pathways in the light of the protective effect of acetate and its donors, it is not unreasonable to suspect that the degree of sensitivity of an animal or its organs to fluoroacetate is an indication of a certain characteristic of its acetate metabolism To establish this suspicion as a fact will require much effort and it is probable that the problem is even more complicated than appears at first Reference has been made to studies with isolated frog muscle, rabbit intestine and intact animals which show that there are fluoroacetate-insensitive metabolic pathways giving rise to sufficient energy to maintain function Evidence has been adduced to indicate that the breakdown of glucose is not sensitive to fluoroacetate and that some such system can be enhanced *in vivo* by partial anoxia (methemoglobinemia) or by accelerating the breakdown with an excess of glucose and insulin It is highly probable that in addition to individual peculiarities of the metabolic systems for handling  $C_1$ - $C_4$  molecules in a given species a second factor, the glycolytic rate, is important in controlling the sensitivity of the organ or organism Few comparative data have been published, although Wu and Chang (134) have recently pointed out that the glycolytic rates of isolated eel, toad, turtle and rat hearts decreased (in that order) with their resistance to anoxia Grossly, the sensitivity to fluoroacetate likewise decreases in the direction of greater glycolytic activity It is also well known that very young mammals are very much more resistant to anoxia than are older ones Nine 24-hour old dogs recently tested in this laboratory were markedly resistant to fluoroacetate Further, the relative sensitivity of various vertebrates to tissue anoxia induced by potassium cyanide (67) is strikingly similar to their order of sensitivity to fluoroacetate Farah (50) has called attention to the similarity

## REFERENCES

1. ABBOTT C E. The effects of DDT and of sodium monofluoroacetate upon *Physarella Oklonka* Morgan. Science 182 71 1945.
2. ALSAUGH, H. Medical Division, Army Chemical Center Private Communication
3. ALBOUT J G. The effect of pH upon the toxicity of iodoacetic acid to yeast cells. J Biol. Chem., 176 83-88, 1948.
4. AXNER, H. S. Metabolism of pyruvic acid in the intact animal. J Biol. Chem., 176 1337-1352, 1948
5. Anon. Compound 1050 as a rodenticide. Brochure prepared by U S Fish and Wildlife Service, 545 Custom House, Denver 2, Colo. (Observation by H. J. Spencer)
6. Anon. Instructions for using sodium fluoroacetate (compound 1050) as a rodent poison. Subcommittee on Mamalogy, National Research Council, Chemical Biological Coordination Center, Washington, D C, Revised October 1943.
7. BACON, J C., BRADLEY C W, HOGEBURG, E. I. TARRANT, P AND CASSIDY J T. Some amides and esters of fluorosuccinic acid. J Am. Chem. Soc. 70 2653-2655, 1948.
8. BACKER, H. J AND VAN MEIS, W. H. Vitesse de la réaction des acides halogéno-carboxyliques et des sulfites. II. Rec. trav. chim. 49: 363-350, 1930.
9. BARBOUR, J H AND SEEVERS M. H. A comparison of the acute and chronic toxicity of carbon dioxide with especial reference to its narcotic action. J Pharmacol and Exper Therap., 78: 11-21, 1943
10. BARCROFT, J MCANALLY R. AND PHILLIPSON, A. Physiological action of acetoacetic acid in living tissues. Nature, 143. 204 1943.
11. BARRON, E. S. G AND MEYER, J. Oxidation of acetate by heart muscle. Proc. Central Soc. Clin. Res., 18 59 1945.
12. BARTLETT G R. AND BARRON, E. S. G. The effect of fluorosuccinate on enzymes and on tissue metabolism. Its use for the study of the oxidative pathway of pyruvate metabolism. J Biol. Chem., 170 67-82, 1947
13. BERGMANN M AND FRUTON, J S. Rockefeller Institute for Medical Research. Private Communication.
14. BIGGLOW, L. A. The action of elementary fluorine upon organic compounds. Chem. Rev., 40 51-115, 1947
15. BLACK, S AND HUTCHINS, J O. The oxidation of acetate by yeast in the presence of fluorosuccinate. Arch. Biochem., 17 211-223, 1948.
16. BLOCH, K. The metabolism of acetic acid in animal tissues. Physiol. Rev. 27 574-620, 1947
17. BOYARSKY L. L., POSTEL, S. AND ROSENBLATT, A. Enzyme inhibitors on conduction and respiration of frog nerve. Fed. Proc. 7 11, 1948.
18. BOYARSKY, L. L., ROSENBLATT, A. D., POSTEL, S. AND GERARD, R. W. Action of methyl fluorosuccinate on respiration and potential of nerve. Am. J. Physiol. 157 291-298, 1949
19. BOTER, P D EVANS, R. J AND PHILLIPS, P H. The toxicity and effect of certain fluorinated derivatives of phenylalanine and tyrosine on the basal metabolic rate of the rat. J Pharmacol. and Exper Therap., 73 176-185 1941.
20. BRAUN MEXENDEL, E. CRUTE, A. L AND GREGORY, R. A. The usage of pyruvic acid by the dog heart. Quart. J Exper Physiol., 29 91-104 1939
21. BATTION S. W AND KLINE, R. F. Age, sex, carbohydrate, adrenal cortex and other factors in anoxia. Am. J. Physiol., 145 190-202, 1945.
22. BROOKS V B AND RANANIEZER, R. E. Enzyme inhibitors on electric activity of frog brain. Fed. Proc., 7 15 1948.
23. BROOKS, V B. RANANIEZER, R. E. AND GERARD, R. W. Action of anticholinesterase drugs and intermediates on respiration and electrical activity of the isolated frog brain. Am. J. Physiol., 157 299-315, 1949
24. BUCKLE, F J HEAP, R. AND SAUNDERS, B. C. Toxic fluorine compounds containing the C-F link. Part III. Fluoroacetamides and related compounds. J. Chem. Soc. 1949 912-918
25. BUCKLE, F J, PATTISON F L M. AND SAUNDERS, B. C. Toxic fluorine compounds containing the C-F link. Part VI.  $\alpha$ -fluorocarboxylic acids and derivatives. J. Chem. Soc., 1949 1471-1479
26. BUKDING E. Studies on the metabolism of the filarial worm, *Loa loa* carinii. J Exper Med., 89 107-130, 1949
27. CEREZIDO L. R. Strain differences in the resistance of rats to pyridoxine deficiency. Fed. Proc., 5 229, 1946.
28. CHENOWETH, M. B AND GILMAN, A. Pharmacology of fluorosuccinate. Fed. Proc., 5 171 1946.
29. CHENOWETH M B AND GILMAN, A. Medical Division, Army Chemical Center Private Communication.
30. CHENOWETH, M. B AND GILMAN, A. Studies on the pharmacology of fluorosuccinate. I. Species response to fluorosuccinate. J Pharmacol. and Exper Therap., 87 90-103, 1945.
31. CHENOWETH M. B AND GILMAN, A. Studies on the pharmacology of fluorosuccinate. II. Action on Heart. Bull. U S Army Med. Dept., 7 687-706, 1947
32. CHENOWETH M. B AND KOELLE, E. S. An isolated heart perfusion system adapted to the determination of non-gaseous metabolites. J Lab. and Clin. Med., 31 600-603, 1946.
33. CHENOWETH, M. B KOELLE E. S., GILMAN A. AND PHILIPS, F S. Unpublished studies 1945.
34. CHENOWETH, M. B AND ST. JOHN E. F. Studies on the pharmacology of fluorosuccinate. III. Effects on the central nervous system of dogs and rabbits. J Pharmacol. and Exper Therap., 89 76-82, 1947
35. CHENOWETH M. B. AND ST. JOHN, E. F. Medical Division, Army Chemical Center Private Communication.
36. CHENOWETH, M. B. SCOTT, E. B. AND SKEL, S. L. Prevention of fluorosuccinate poisoning by acetate donors. Fed. Proc. 8 280 1949
37. CHENOWETH, M. B., JOHNSON L. B., KANDEL, A., SCOTT E. B. AND WEEKS, J. R. Work in progress.
38. CLARKE D A. TOOLE, J F AND RIKER, W F JR. Effect of fluorosuccinate on oxidative processes in frog muscle. Fed. Proc. 8 282 1949
39. CLARKE D A. AND RIKER, W F, JR. To be published.
40. CONER, B, Johns Hopkins University Private Communication.

phenomenon, however, is outstandingly unexplained. If blockades by the higher fluoroacids actually occur as indicated, why are these acids so much more toxic? Perhaps this will become clear as the understanding of fatty acid metabolism increases. On the whole, it seems possible to explain the pharmacological actions of fluoroacetate itself, together with some of the specific variations noted, as a function of the magnitude and character of the acetate and glycolytic metabolic pathways. The characteristic response of an organism or tissue to fluoroacetate may be determined by the relative importance of these two pathways.

#### SUMMARY

Conversion of a metabolic intermediate into a very highly toxic compound by the introduction of a single fluorine atom in a strategic position in the molecule has been described for a number of compounds. It appears to be a useful method for producing agents with which metabolic pathways can be differentiated in a large number of species with a minimum of effort, for it is evident that these agents act by virtue of their close resemblance to natural metabolites. As an example, the variation among species in response to monofluoroacetic acid has been related to certain definite differences in metabolism in the species studied.

#### ACKNOWLEDGMENTS

The author wishes to thank the Office of the Chief, Chemical Corps, for permission to use hitherto unpublished information, and Mrs Pauline Koepnick and the Editors for aid in the preparation of the manuscript. Dr Walter Kirner aided greatly in obtaining declassification.

#### ADDENDUM

The gracious permission of the British Ministry of Supply to refer to information in certain of their classified reports was received too late to permit inclusion of this information in the body of the review. Mention should be made, however, of the high resistance of the *Cercopithecus*, or "green" monkey to methyl fluoroacetate. The  $LD_{50}$  appears to be above 50 mgm/kgm according to K. J. Carpenter and B. A. Kilby (1944). These workers also found that although fluoroethanol is as active as fluoroacetate in intact animals, it is without action on the isolated perfused heart, presumably because this organ can not convert fluoroethanol to fluoroacetate.

Definite histologic abnormalities in the myocardium, but no unequivocal changes in the central nervous system, were reported by A. M. Barrett (1944) in guinea pigs and rabbits poisoned repeatedly with methyl fluoroacetate. The beating of heart muscle cells in culture is rapidly inhibited by methyl fluoroacetate but the growth of the cell masses is not affected, according to C. B. Allsop and H. B. Fell (1944). The action is specific, for methyl chloroacetate in equivalent concentrations merely kills the cells.

The view that fluoroacetic acid is metabolized to fluorocitric acid, accounting for inhibition of citrate oxidation and the subsequent accumulation of citric acid, has been advanced by Martius (Ann. d. Chem. 561: 227-232, 1949). This would seem referable to Blockade (C) of Figure 1.



- 82 KRAHL, M. E. AND CORI, C. F. The uptake of glucose by the isolated diaphragm of normal, diabetic and adren-  
ectomized rats. *J Biol. Chem.*, 170 607-619, 1947
- 83 LELOIR, L. F. The mechanism of fatty acid oxidation. *Enzymologia*, 17 263-276, 1948
- 84 LIÉBECQ, C. AND PETERS, R. A. The toxicity of fluoracetate and the tricarboxylic cycle. *Biochimica et Bio-  
physica Acta*, 3 15-230, 1949
- 85 LIPTON, M. A. Mechanism of the enzymatic synthesis of acetylcholine. *Fed. Proc.*, 5 145, 1946.
- 86 LITZKA, G. Allgemeines biologische Wirkungen einer kernfluorierten Aminosäure (Fluortyrosin) *Arch. f  
exper Path. u Pharm.*, 183 427-435 1935.
- 87 LITZKA, G. Die antithyretotische Wirkung des Fluortyrosin *Arch. f exper Path. u Pharm.*, 183. 436-453, 1936
- 88 LUND, A. Metabolism of methanol and formic acid in rabbits. *Acta Pharmacol. et Toxicol.*, 4 99-107, 1943
- 89 LUND, A. Metabolism of methanol and formic acid in dogs. *Acta Pharmacol. et Toxicol.*, 4 103-121, 1943
- 90 LUND, A. Excretion of methanol and formic acid in man after methanol consumption. *Acta Pharmacol. et  
Toxicol.*, 4. 205-212, 1943
- 91 LYNCH, F. Zum biologischen Abbau der Essigsäure I Über die "Induktionzeit" bei verarmter Hefe. *Ann d  
Chem.*, 552- 270-306, 1942.
- 92 MACCHIARELLO, A. Plague control with DDT and "1050" Results achieved in plague epidemic at Tumbes  
Peru, 1945 *Am. J Pub Health*, 36: 842-854, 1945.
- 93 MAMAR, J. S. C. Monofluoroacetic acid, the toxic principle of "Gifblaar," *Dichapetalum cymosum* (Hook) Engl  
Onderstepoort *J Vet. Sci. Animal Ind.*, 20 67-73, 1944.
- 94 MAJUR, A., BODANSKY, O. AND ALBAUM, H. Medical Division, Army Chemical Center Private Communication
- 95 MAJUR, A. BODANSKY, O. AND ALBAUM, H. Ibid.
- 96 McCOMBIE, H. AND SAUNDERS, B. C. Fluoroacetates and allied compounds. *Nature*, 158: 332-335, 1946.
- 97 McNAMARA, B. P. Medical Division, Army Chemical Center Private Communication.
- 98 MICHAELIS, M., ARANGO, N. I. AND GERRARD, R. W. Inhibition of brain dehydrogenases by anticholinesterases  
*Am. J Physiol.*, 157: 463-467, 1949
- 99 MODELL, W., KOSTER, R. AND RIEKE, W. F., JR. Cornell University Medical College. Private Communication.
- 100 MORRISON, J. L. Toxicity of certain halogen substituted aliphatic acids for white mice. *J Pharmacol. and Exper  
Therap.*, 86 336-338, 1946
- 101 Numerous Authors. A fluorine symposium. *Ind. Eng. Chem.*, 39: 235-436, 1947
- 102 PRICE, C. C. AND JACKSON, W. G. Some properties of methyl fluoroacetate and fluoroethanol. *J Am Chem  
Soc.* 69: 1065-1066, 1947
- 103 PAULING, L. The Nature of the Chemical Bond. Cornell University Press, Ithaca, N. Y., 1939
- 104 PAULING, L. AND HUGGINS, M. L. Covalent radii of atoms and interatomic distances in crystals containing elec-  
tron-pair bonds. *Ztschr f Krist.* 87: 203-238, 1934.
- 105 QUINT, J. I. AND CLARK, R. Studies on the action of potassium monofluoroacetate ( $\text{CH}_3\text{FCOOK}$ ) *Dichapetalum  
cymosum* (Hook) Engl toxin on animals. Onderstepoort *J Vet. Sci. Animal Ind.*, 22: 77-90, 1947
- 106 RAMEY, L. L. AND CLIFFORD, P. A. Determination of monofluoroacetic acid in foods and biological materials.  
Presented at the May 12 1949 meeting of the Washington Section of the American Chemical Society
- 107 RENSHAW, B. AND GATES, M. National Defense Research Council. Private Communication
- 108 ROHMOLD, K. Fluor and Fluorverbindungen, in Heffter's Handbuch der Experimentellen Pharmakologie VII  
1938.
- 109 SAUNDERS, B. C. Toxic properties of  $\alpha$ -fluorocarboxylic acids and derivatives. *Nature*, 160 179-181, 1947
- 110 SAUNDERS, B. C. AND STACEY, G. J. Toxic fluorine compounds containing the C-F link. Part I. Methyl fluoro-  
acetate and related compounds. *J Chem. Soc.*, 1948 1773-1779
- 111 SAUNDERS, B. C. AND STACEY, G. J. Toxic fluorine compounds containing the C-F link. Part IV (a) 2-fluoroethyl  
fluoroacetate and allied compounds. (b) 2' 2' difluorodithiyl ethylene dithiolyol. *J Chem. Soc.*, 1949 916-919
- 112 SAUNDERS, B. C., STACEY, G. J. AND WILDING, I. G. E. Toxic fluorine compounds containing the C-F link. Part  
II 2 Fluoroethanol and its derivatives. *J Chem. Soc.* 1949 773-777
- 113 SAUNDERS, B. C. AND WILDING, I. G. E. Toxic fluorine compounds containing the C-F link. Part V (a) Fluorine-  
containing ammonium salts. (b) Relationship between physiological action and chemical constitution. *J Chem.  
Soc.* 1949 1279-1282.
- 114 SCALES, J. W. "1050" rat poison very effective but dangerous later *Farm Research*, 8 No. 12, p 8, 1945
- 115 SCHWARTZ, L. H. Toxicity of sodium monofluoroacetate (1050) for swine and chickens. *J Am. Vet. Med.  
Assoc.*, 111 301-303 1947
- 116 SHANES, A. Action of methyl fluoroacetate on metabolic processes sustaining resting potential of frog nerve.  
Rockefeller Institute for Medical Research. Private Communication.
- 117 SHANES, A. AND BROWN, D. E. J. The effect of metabolic inhibitors on the resting potential of frog nerve. *J Cell  
and Comp Physiol.* 19 1-13, 1942.
- 118 SIDGWICK, V. V. The Covalent Link in Chemistry Cornell University Press, Ithaca New York, 1933
- 119 SIMMONS, S. W. AND NICHOLSON, H. P. Use of the rodenticides 1050 and ANTU *Pests* 15 Nos. 5 8 10, 1947
- 120 SMITH, P. K. GLEASON, H. L., STOLL, C. G. AND OGORZALEK, S. Studies on the pharmacology of salicylates  
*J Pharmacol. and Exper Therap.* 87 237-255, 1945
- 121 STADIE, W. C. The intermediary metabolism of fatty acids. *Physiol. Rev.* 25 395-441 1945
- 122 SWARTZ, F. Sur l'acide fluoracétique. *Bull. Soc. Chim. de Paris I Série* 3, 15 1134, 1896.
- 123 TEPPERMAN, J. AND MAJUR, A. Personal communication 1945.
- 124 TOENIESSEN, E. AND BRINKMANN, E. Über den Abbau der niederen Fettsäuren insbesondere der Essigsäure  
und Ameisensäure im Säugtier und über die Frage der Zuckerbildung aus Fett *Z physiol. Chem.*, 251- 169-  
195 1936

- 41 COLOWICK, S P, BERGER, L, SLEIN V W AND CORI, C F Washington University St. Louis. Private Communication
- 42 COTTRAL, C E, DIBBLE, G D AND WINTON, B Effect of sodium fluoroacetate ("1080" rodenticide) on White Leghorn chickens Poultry Sci, 26 610-612, 1947
- 43 DEONIER, C C, JONES, C A AND INCHO, H H Organic compounds effective against *Anopheles quadrimaculatus* Laboratory tests J Econ Entomol, 39 459-462, 1946
- 44 DIEHL, S H AND RICHTER, C P Comparative assays of rodenticides on wild Norway rats. I. Toxicity U S Pub Health Repts, 61 672-679, 1946
- 45 DOTY, R W AND GERARD, R W Separate inhibition of resting and active oxygen consumption of functioning nerves Fed Proc, 8 35, 1949
- 46 EISENBERG, W V AND WILSON, J B Identification of the monohalogen substituted acetic acids as barium salts J Assoc Offic. Agricul Chemists, 30: 563-567, 1947
- 47 ELLIOTT, A M Personal Communication
- 48 ELMORE, J W Report on the analysis of rodenticides J Assoc. Offic. Agricul Chemists 31: 366-368, 1948
- 49 FARAH, A., AMOEL, R AND WEST, T C Action of some enzyme inhibitors on the activity of the isolated intestine Fed Proc. 8 290, 1949 J Pharmacol. and Exper Therap In press
- 50 FOLLEY, S J AND FRENCH, T H Acetate as a possible precursor of ruminant milk fat, particularly the short chain fatty acids. Nature 163 174-175, 1949
- 51 FOSS, G L. The toxicology and pharmacology of methyl fluoroacetate (MFA) in animals, with some notes on experimental therapy Brit. J Pharm and Chemother, 3: 118-127, 1948
- 52 FRICK, E J AND BOEBEL, F W Clinical observation of "1080" poisoning Veter Med, 41 196-197, 1946
- 53 FURCHOTT, R F To be published
- 54 FURCHOTT, R. F AND SHOEN, E Sources of energy for intestinal smooth muscle contraction Proc. Soc. Exper Biol and Med., 61: 280-285, 1948.
- 55 GLOMSET, D J AND GLOMSET, A T A morphologic study of the cardiac conduction system in ungulates, dog and man Am Heart J, 20 677-701, 1940
- 56 GOLDMAN, I S., FLANNERY, M C, ARENT, L J, HOAG, J B AND BUEWELL, A M Medical Division, Army Chemical Center Private Communication
- 57 GRATCH, I, PURLIA, P L AND MARTIN, M L. Effect of sodium fluoroacetate (1080) in poisoned rats on plague diagnosis procedures. Preliminary report. U S Pub Health Repts., 64 339-342, 1949
- 58 GRIMM, H G AND WOLF, H in GERDNER, H AND SCHULZ, K. Handbuch der Physik, 2nd Edition, 24: 1003, 1933.
- 59 GRYKIEWICZ TROCIMOWSKI, E, SPORYNSKI, A. AND WNUK, J Recherches sur les composés organiques fluorés dans la série aliphatique I-IV Res trav chim, 66 413-431, 1947
- 60 HENNE, A L Aliphatic fluorides Chapter XI, in Gilman, H., Organic Chemistry, An Advanced Treatise, Vol I, 2nd Ed, Wiley, New York, 1943
- 61 HINWICH, H. E Medical Division, Army Chemical Center Private Communication
- 62 HÖRAN, R Physical Chemistry of Cells and Tissues, Blackiston, Philadelphia, 1945 Chapter 12.
- 63 HOFF, E C AND FULTON, J F, A bibliography of aviation medicine, C C Thomas and Co., 1942 and Supplement, G Banta Publ Co., Menasha, Wisc, 1944
- 64 HORSFALL, J L 2-ethyl hexyl fluoroacetate as aphid poison U S Patent 2,409 859, October 22, 1946
- 65 HUQUES, J H Densitisation of surface vessels by means of 1080 (sodium fluoroacetate) U S Pub Health Repts., 62 933-940, 1947
- 66 HUNT, R. Cyanwasserstoff, in Hefter's Handbuch der Experimentellen Pharmakologie I, pp 772-773, 1923
- 67 HUTCHENS, J O AND KASS, B M A colorimetric microanalytical method for acetate and fluoroacetate J Biol Chem, 177: 571-575, 1949
- 68 HUTCHENS, J O, McMAHON, T AND PODOLSKY, B University of Chicago Toxicity Laboratory Private Communication
- 69 HUTCHENS, J O, WAONER, H, PODOLSKY, B AND McMAHON, T M The effect of ethanol and various metabolites on fluoroacetate poisoning. J Pharmacol and Exper Therap, 95: 62-70, 1949
- 70 HYDE, J, BECKETT, S. AND GELTHORN, E Acetylcholine and convulsive activity J Neuropsychol. 12 17-37, 1949
- 71 I G Farbenindustrie, A. G German patent 504,886, September 14, 1927, confirmed August 8, 1930 (Through Bockemüller, W Organische Fluorverbindungen in Sammlung chemischer und chemisch-technischer Vorträge, No 28, 89 Stuttgart, 1938.)
- 72 JEMKINE, R. L. AND KOEHLER, H. C Making 1080 safe A case study in the safe manufacture and distribution of a hazardous chemical Chem. Ind., 62 232-233, 1945
- 73 KALINICKI, E. R. "Ten Eighty," A war produced rodenticide. Science, 102 232-233, 1945
- 74 KALINITSKY G The effect of BaCl<sub>2</sub> MgCl<sub>2</sub> and fluoroacetate on the formation and utilization of citrate Arch. Biochem, 17: 403-411, 1948.
- 75 KALINITSKY G The formation of citrate by extracts of rabbit kidney cortex. J Biol Chem, 179: 1015-1023, 1949
- 76 KALINITSKY, G AND BARRON, E S G The effect of fluoroacetate on the metabolism of yeast and bacteria. J Biol Chem, 170 83-95 1947
- 77 KALINITSKY, G AND BARRON, E S G The inhibition by fluoroacetate and fluorobutyrate of fatty acid and glucose oxidation produced by kidney homogenates. Arch Biochem 19: 75-87, 1946
- 78 KEMPF, C A, GREENWOOD, D A AND NELSON V E Studies relating to the toxicity of fluorine compounds. J Lab and Clin Med, 22: 1133-1137 1947
- 79 KREIBACH M S University of Chicago Private Communication.
- 80 KING, J E AND PENFOUND, W T Effects of new herbicides on fish. Letter to the Editor Science, 103 437 1946.

82. KRAHL, M. E. AND COBI, C. F. The uptake of glucose by the isolated diaphragm of normal, diabetic and adren-  
alectomized rats. *J. Biol. Chem.*, 170: 607-618, 1947
83. LELAND, L. F. The mechanism of fatty acid oxidation. *Enzymologia* 12: 263-276, 1948
84. LÉFEBVRE, C. AND PETERS, R. A. The toxicity of fluoroacetate and the tricarboxylic cycle. *Biochimica et Bio-  
physica Acta*, 3: 15-230, 1949
85. LIPPON, M. A. Mechanism of the enzymatic synthesis of acetylcholine. *Fed. Proc.*, 5: 145, 1946.
86. LITZKA, G. Allgemeines biologische Wirkungen einer kernfluorierten Aminosäure (Fluortyrosin). *Arch. f.  
exper. Path. u. Pharm.* 183: 427-435, 1936
87. LITZKA, G. Die antithyretotoxische Wirkung des Fluortyrosin. *Arch. f. exper. Path. u. Pharm.*, 183: 436-453, 1936
88. LUND, A. Metabolism of methanol and formic acid in rabbits. *Acta Pharmacol. et Toxicol.*, 4: 99-107, 1948
89. LUND, A. Metabolism of methanol and formic acid in dogs. *Acta Pharmacol. et Toxicol.*, 4: 103-121, 1948
90. LUND, A. Excretion of methanol and formic acid in man after methanol consumption. *Acta Pharmacol. et  
Toxicol.*, 4: 205-212, 1948
91. LYNN, F. Zum biologischen Abbau der Essigsäure I. Über die "Induktionszeit" bei verarmter Hefe. *Ann. d.  
Chem.*, 553: 270-306, 1942
92. MACCHIARELLO, A. Plague control with DDT and "1050" Results achieved in plague epidemic at Tumbes  
Peru, 1945. *Am. J. Pub. Health*, 35: 842-854, 1945
93. MARAIS, J. S. C. Monofluoroacetic acid, the toxic principle of "Gifblaar" *Dichapetalum cymosum* (Hook) Engl  
Onderstepoort *J. Vet. Sci. Animal Ind.*, 20: 67-73, 1944.
94. MASUR, A., BODANSKY, O. AND ALBAUM, H. Medical Division, Army Chemical Center. Private Communication
95. MASUR, A., BODANSKY, O. AND ALBAUM, H. *Ibid.*
96. McCOMBIE, H. AND SAUNDERS, B. C. Fluoroacetates and allied compounds. *Nature*, 159: 382-385, 1946.
97. McNAMARA, B. P. Medical Division, Army Chemical Center. Private Communication.
98. MICHAELIS, M., ARANGO, N. L. AND GERARD, R. W. Inhibition of brain dehydrogenases by anticholinesterases  
*Am. J. Physiol.*, 157: 463-467, 1949
99. MORRIS, W., KOSTER, R. AND REIKES, W. F., JR. Cornell University Medical College. Private Communication.
100. MORRISON, J. L. Toxicity of certain halogen substituted aliphatic acids for white mice. *J. Pharmacol. and Exper  
Therap.*, 86: 336-338, 1946.
101. Numerous Authors. A fluorine symposium. *Ind. Eng. Chem.*, 39: 235-436, 1947
102. PRICE, C. C. AND JACKSON, W. G. Some properties of methyl fluoroacetate and fluoroethanol. *J. Am. Chem  
Soc.*, 69: 1065-1068, 1947
103. PAULING, L. The Nature of the Chemical Bond. Cornell University Press Ithaca, N. Y., 1939
104. PAULING, L. AND HUGGINS, M. L. Covalent radii of atoms and interatomic distances in crystals containing elec-  
tron pair bonds. *Ztschr. f. Krist.* 87: 205-233, 1934.
105. QUIN, J. I. AND CLARK, R. Studies on the action of potassium monofluoroacetate ( $\text{CH}_3\text{FCOOK}$ ) *Dichapetalum  
cymosum* (Hook) Engl. toxin on animals. Onderstepoort *J. Vet. Sci. Animal Ind.*, 22: 77-90, 1947
106. RAMSEY, L. L. AND CLIFFORD, P. A. Determination of monofluoroacetic acid in foods and biological materials.  
Presented at the May 12, 1949 meeting of the Washington Section of the American Chemical Society
107. RENSHAW, B. AND GATES, M. National Defense Research Council. Private Communication
108. ROHM, H. Fluor und Fluorverbindungen, in *Hefters Handbuch der Experimentellen Pharmakologie VII*  
1938
109. SAUNDERS, B. C. Toxic properties of  $\alpha$ -fluorocarboxylic acids and derivatives. *Nature* 160: 179-181, 1947
110. SAUNDERS, B. C. AND STACEY, G. J. Toxic fluorine compounds containing the C-F link. Part I. Methyl fluoro-  
acetates and related compounds. *J. Chem. Soc.*, 1948: 1773-1779
111. SAUNDERS, B. C. AND STACEY, G. J. Toxic fluorine compounds containing the C-F link. Part IV. (a) 2-fluoroethyl  
fluoroacetate and allied compounds. (b) 2,2'-difluorodimethyl ethylene dithioglycol. *J. Chem. Soc.* 1949: 916-919
112. SAUNDERS, B. C., STACEY, G. J. AND WILDING, I. G. E. Toxic fluorine compounds containing the C-F link. Part  
II. 2-fluoroethanol and its derivatives. *J. Chem. Soc.*, 1949: 773-777
113. SAUNDERS, B. C. AND WILDING, I. G. E. Toxic fluorine compounds containing the C-F link. Part V. (a) Fluorine-  
containing ammonium salts. (b) Relationship between physiological action and chemical constitution. *J. Chem.  
Soc.* 1949: 1279-1282.
114. SCALES, J. W. "1050" rat poison very effective but dangerous later. *Farm Research*, 8, No. 12, p. 8, 1945.
115. SCHWARTZ, L. H. Toxicity of sodium monofluoroacetate (1050) for swine and chickens. *J. Am. Vet. Med.  
Assoc.* 111: 301-303, 1947
116. SHANLEY, A. Action of methyl fluoroacetate on metabolic processes sustaining resting potential of frog nerve.  
Rockefeller Institute for Medical Research. Private Communication.
117. SHANLEY, A. AND BROWN, D. E. J. The effect of metabolic inhibitors on the resting potential of frog nerve. *J. Cell  
and Comp. Physiol.* 19: 1-12, 1942.
118. SLOCUM, N. V. The Covalent Link in Chemistry. Cornell University Press Ithaca, New York, 1933
119. SIMMONS, S. W. AND NICHOLSON, H. P. Use of the rodenticides 1050 and ANTU. *Pests* 15: Nos. 5 & 10, 1947
120. SMITH, P. K., GLEASON, H. L., STOLL, C. G. AND OGONZALEZ, S. Studies on the pharmacology of salicylates.  
*J. Pharmacol. and Exper. Therap.*, 87: 237-255, 1945.
121. STADIE, W. C. The intermediary metabolism of fatty acids. *Physiol. Rev.* 25: 395-441, 1945
122. SWARTS, F. Sur l'acide fluoroacétique. *Bull. Soc. Chim. de Paris I. Série* 3, 15: 1134, 1928
123. TEPPERMAN, J. AND MAZUR, A. Personal communication, 1945
124. TOENIESSEN, E. AND BRENNEMANN, E. Über den Abbau der niederen Fettsäuren insbesondere der Essigsäure  
und Ameisensäure im Säugetier und über die Frage der Zuckerbildung aus Fett. *Z. physiol. Chem.*, 252: 169-  
195, 1925

125. TOURTELLOTT, W W AND COON, J M Synergistic effect of sodium acetate and ethanol in antagonising sodium fluoroacetate (1080) poisoning in mice. Fed. Proc. 8 239 1949
126. WARD A A. Convulsive activity induced by fluoroacetate. J Neurophysiol., 10: 105-112, 1947
127. WARD, J C Personal communication, 1944.
128. WARD, J C Rodent control with 1080, ANTU and other war-developed toxic agents. Am. J. Pub. Health, 36: 1427-1431, 1946.
129. WARD, J C. AND SPENCER, D A. Notes on the pharmacology of sodium fluoroacetate-Compound 1080. J Am. Pharm. Assoc., 36: 59-62, 1947
130. WATSON, H B Modern Theories of Organic Chemistry Oxford University Press, London, 1947, p. 36.
131. WATT J M AND BREYER BRANDWILK M G The Medicinal and Poisonous Plants of South Africa. Livingston Co, Edinburgh, 1932, pp 97-98.
132. WEEKS, J R. AND CHENOWETH M B Separation by fluoroacetate of energy sources for smooth muscle contraction. Fed Proc, 8. 345, 1949
133. WOODS, L A Personal communication, 1949
134. WU J J AND CHANG, I The glycolytic activity of the hearts of vertebrates. Quart. J Exper Physiol., 34: 91-95, 1948.

# THE EFFECTS OF DRUGS UPON THE ELECTRICAL ACTIVITY OF THE BRAIN\*

JAMES E P TOMAN AND JEAN P DAVIS

*Departments of Physiology and Pharmacology, University of Utah College of Medicine,  
Salt Lake City, Utah*

## I INTRODUCTION

It is now twenty years since Hans Berger (20) presented to the world a revolutionary new method for the investigation of the function of the brain in health and disease, namely, *electroencephalography*. His contribution was threefold. To the clinician he gave a diagnostic instrument with sharp differential and localizing value, without which the notable advances of the last two decades in the pharmacological and surgical treatment of central nervous disorders would have been sorely handicapped. To the neurophysiologist he gave a research tool of great precision and relative simplicity for the unraveling of the central nervous pathways by the monitoring of their electrical signs. Lastly, to all those who strive to understand the mode of operation of the organ of thought, he gave a new point of departure. The brain could no longer be considered a passive switch-board through which impulses coursed on their way to and from the periphery, it was now revealed as a dynamic participant in the affairs of the body, possessing an inherent spontaneous and rhythmic activity which could modify the soma and be modified in turn by the soma.

Berger did not neglect to investigate the effects of drugs upon the electrical activity of the brain (21). He looked for electroencephalographic signs of the central nervous actions of barbital, morphine, cocaine, amyl nitrite, scopolamine, chloroform and other substances then in common clinical use. Thus he opened a field of research which has many potentialities not only for the determination of the mechanism of action of centrally acting drugs, but also for the analysis of the nature of the electroencephalogram itself, for the reason that substances with known specific action are among the most precise tools available for investigations in the biological sciences.

Unfortunately, the rapidly increasing volume of literature in the field of electroencephalography contains relatively few systematic studies of the effects of drugs on the electrical activity of the brain, although there are many empirical reports on the alterations produced by particular chemical agents. Among previous reviews relevant to this subject should be mentioned those of Hayslip (176), Gibbs and Gibbs (135), Finesinger and Brazier (108), Gibbs (130, 131, 132), Lennox and Lennox (217), and Walter and Walter (331).

The present review will consider primarily the effects of drugs upon the normal human EEG. This will necessarily exclude from discussion most of the large

\*Original investigations and other work incident to the preparation of this review were supported in part by grants in-aid of research from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

body of clinical reports containing casual observations of drug alterations of the EEG in convulsive disorders, cerebral trauma, etc. Experimental studies in animals will be included to the extent that they contribute to an understanding of drug actions or of the neurophysiological basis of the recorded electrical activity of the brain. Only the more relevant work dealing with topical application of drugs to the brain will be evaluated. Finally, priority will be given to a few selected agents which are either of heuristic value or commonly used in the treatment of disease. In atonement for this cavalier treatment of a large section of the electroencephalographic literature, the reviewers hope to emerge with a few examples of the usefulness and limitations of the EEG as an aid to the understanding of the mechanism of action of drugs upon the central nervous system, and also of drugs as analytical tools for investigating the nature of the EEG.

## II ON THE GENESIS OF THE ELECTROENCEPHALOGRAM

Any rational discussion of the mechanism of action of drugs upon the EEG presupposes some knowledge of the nature of the electrical discharges recorded from the brain. Unfortunately this body of information is still in the most primeval state, and consequently the most divergent extremes of interpretation persist in the literature (cf 331). Thus the EEG is viewed by some investigators as the synchronized summation of large numbers of action potential spikes (2), by others, as slow fluctuations in the somatic membrane potential of cortical neurones (43, 127). Some view the rhythmic activity as an inherent property of the individual neurones (142), while others emphasize the importance of cyclic reverberations in closed neuronal chains (26). Some regard the grossly recorded EEG as a mixture of sinusoidal oscillations (62), others, as a composite of discrete non-sinusoidal pulses (2). Functionally, the dominant resting cortical rhythms have been viewed as merely fortuitous synchronizations of groups of cortical neurones (2), as a mechanism of alternating excitation and inhibition, which tends to regulate the overall excitability of the cortex (42), as a spatial scanning device for the comparison of new and stored information (245, 276), or as an escapement system limiting cortical output to fixed time intervals (71, 72). If the reviewers seem somewhat partisan in their attitude toward these various controversies, it is only to maintain a reasonable working hypothesis against which the observed actions of drugs may be projected.

First, with regard to the wave composition of the EEG, although much of the clinical literature implies a mixture of sinusoidal oscillations (cf 135) and attempts have been made to synthesize even the abnormal paroxysmal discharges from a summation of pure frequency components (62), this conception may be somewhat misleading. By analogy, the gross electromyogram of a muscle which contains many discharging motor units may give the appearance of a mixture of pure frequencies, but finer analysis reveals the existence of discrete and characteristic action potential spikes. Unfortunately, the recording of spontaneous single unit discharges in the brain is technically most difficult. Renshaw *et al* (279) have recorded such unit action potentials from the olfactory cortex, Moruzzi *et al* (259) from Purkinje cells of the cerebellum, and Adrian and

Moruzzi (3) indirectly from pyramidal cells of the motor cortex. In general these studies show discrete spikes rather than an underlying sinusoidal rhythm.

Even with gross recording of the electrical activity of the brain, one cannot but be impressed with the amount of fine detail submerged in the overt sinusoidal rhythm. For example, the so-called "Dial" potentials (60, 88), recorded from rostral areas of animals under light barbiturate anesthesia and resembling "sleep spindles" (75) in man, are complex in form even when recorded with scalp electrodes, when led off from pial electrodes, they may be observed as discrete repetitive diphasic or polyphasic responses, in which the several components may be independently modified by drug action (317).

A useful method for analysis of the EEG is the evocation of synchronized discharges from brain by stimulation of sensory nerves and organs or of central tracts. The best such studies in anesthetized experimental animals reveal early spike components which may be attributed to arriving tract impulses, monosynaptic transmission, and the activation of subsequent banks of synapses (69, 264). They may also be seen in the electrocorticogram of unanesthetized animals (244, 346). Even the firing of a single bank of cells gives a complex response because of the different time characteristics of dendrites, somata and axones (239, 264). The initial sequence of spike-like events may be followed by one or more waves having in general the time course and spatial distribution of the characteristic "spontaneous" waves arising in the same locus at rest. They appear to originate from the entire thickness of the cortex (3, 264). In appearance they approximate the spontaneous EEG at all levels of central responsiveness from deep anesthesia to the subconvulsive state, but the evoked records preserve a discreteness and reproducibility which is not as evident in the spontaneous activity, probably because of the greater dispersion of the latter (315).

From the above considerations it is evident that the "spontaneous" electroencephalogram of a brain receiving impulses from sensory or other sources must in part represent the summation of many unit discharges, varying in their time course from spikes of the order of a millisecond to slow waves of 100 milliseconds or more. If these were completely random in occurrence, no observable rhythms would be grossly recorded in the EEG. If they were completely synchronized in homogeneous brain areas, much of the fine detail seen in the evoked discharges could be observed with a suitably rapid recording system. The true state of affairs would appear to lie intermediate between the extreme states of dispersion and synchrony. In this intermediate state it is evident that partial dispersion will attenuate the fast more than the slow components in the gross EEG record.

Secondly, as to the possibility of an inherent rhythmicity of cerebral cells, the respiratory "center" was at one time exploited as an analog of inherently spontaneous cerebral rhythms (135). More recently, the work of Pitts, Magoun and Ranson (275) has revealed the role of bulbar and peripheral inhibitory feedback circuits in the maintenance of the respiratory cycle, and concurrently the role of reverberating circuits in maintaining cortical rhythms has been emphasized. To complete the cycle, current evidence now indicates the possibility that with a

suitable chemical drive the isolated inspiratory center may act as a relaxation oscillator (195). In peripheral nerve, spontaneous rhythms having a slower time course than action potential spikes are well known (8, 47, 238). The cerebral cortex deprived of all afferent connections (40) or so treated by drugs as to render synaptic transmission presumably impossible (230) may continue to show rhythmic activity. Thus it is not impossible that non-propagated fluctuations in neuronal membrane potential may contribute to the EEG. This in no way diminishes the significance of reverberating circuits such as those which have been postulated for cortico-thalamic (26) and cortico-hypothalamic (263) interaction. Since oscillations in membrane potential tend to be associated with cyclic changes in excitability of neurones, giving rise to propagated spikes when the fluctuations are sufficiently great (47), and since the local oscillations themselves can be intensified and synchronized by arriving propagated impulses (238), it is conceivable that both the local periodicity and the total time delay of a reverberating pathway may enter into the determination of the frequency of a recorded rhythm in an active and transmitting brain.

Finally, as to the functional significance of the EEG, certain generalizations must be borne in mind, particularly in evaluating drug action. Although the dominant alpha rhythm is commonly considered an indicator of the resting wakeful state, it is obvious that many subjects at rest and almost all with eyes open show only a highly desynchronized and irregular low-voltage fast activity, which is also typical of most unanesthetized animals in the waking state. Furthermore, it is common clinical experience that consciousness and other attributes of the functionally normal brain may persist in patients with bizarre diffuse dysrhythmias, either of pathological origin or pharmacologically induced. However, there are extreme EEG states in which higher function is grossly impaired, such as the hypersynchronized hyperdischarge of the tonic-clonic and the petit mal seizure, the hypersynchrony of moderate barbiturate anesthesia, or the complete inactivity characterizing very deep anesthesia or post-seizure depression. Thus the ability of the brain to receive, store and transmit information is not incompatible with a wide range of EEG patterns, except at the extremes of gross diffuse hypersynchronization, hyperactivity or complete inactivity.

### III SOME HYPOTHETICAL MECHANISMS OF ACTION OF DRUGS UPON THE EEG

Having disposed of these preliminary non-pharmacological considerations of the complex origin of the EEG, one can now appropriately inquire into the known mechanisms by which drugs may alter the properties of the grossly recorded electrical activity of the brain. The effects of drugs upon the properties of neurones will be the subject of a subsequent review (318), and only brief mention of some of the possible alterations will be attempted here.

Among the passive properties of nerve cells, which can be measured without the elicitation of a test response, should be included the membrane resistance, capacity, inductance and voltage. The capacity and inductance are not known to be easily modified by drug action, in the presence of a suitably low damping membrane resistance, they may determine the frequency of cyclic oscillations in



the membrane potential (47) Together with the membrane and longitudinal resistances they enter into the determination of the spatial and temporal characteristics of excitation and response (238) Membrane resistance is known to be reduced by potassium ion (193), and perhaps may be increased in central neurones by the barbiturates (93) Membrane voltage must be maintained above a critical level to provide a resting, excitable cell Since the voltage is dependent upon metabolic work, it is subject to alteration by a wide variety of metabolic agents as well as by respiratory gas tensions and the electrolyte pattern of the extracellular fluid, its separable components, which enter into the excitation and response characteristics of nerve cells, may be independently modified by drug action (238)

Among the active properties of neurones are those concerned with excitation the threshold, or critical potential to which membrane potential must be reduced in order to initiate a propagated discharge, the temporal and spatial requirements for excitation, and the rate and extent of accommodation, or the change in threshold with applied stress Relatively little is known about drug effects on these properties, with the exception of threshold, which by gross methods is generally but not always found to be elevated by depressants and reduced by excitants Changes in cerebral cortical threshold are not necessarily reflected in EEG alterations, for example, in experimental animals the threshold may be considerably raised by benzimidazole with no change in EEG (154), and wide spontaneous fluctuations in cortical threshold may be seen without corresponding EEG manifestations, all of which would seem to indicate that the stability and frequency of cortical rhythms are insured by regulating mechanisms which correct for threshold alterations within wide limits

A second set of neuronal properties are those relevant to the response, these include the form, duration and amplitude of the action potentials and the velocity with which they are propagated, as well as the tendency toward spontaneous discharges and the occurrence of high frequency trains of spikes instead of isolated unit discharges Again little is known of these aspects of responsiveness in cerebral neurones, except insofar as high frequency discharges appear to be associated with convulsive responses, may be elicited by various convulsant drugs, and are reflected in the gross EEG as high voltage spikes (3, 258)

Finally, there are those phenomena associated with recovery following antecedent stimulation, these include the absolute and relative refractory periods and the supernormal and subnormal phases of recovery of excitability, as well as oscillatory phenomena in the recovery cycle It might be expected that in an active region of brain any prolongation of the recovery cycle would have profound effects on the EEG, particularly in the direction of slowing spontaneous rhythms, indeed, this seems to be the case at least with the barbiturates (198, 243) Also there must be included the long periods of facilitation seen after excessive stimulation of peripheral nerve, which may be abolished by anticonvulsant drugs and which have been assumed to play a role centrally in the development and spread of seizures (317)

In addition to those properties which characterize neurones in isolation, there

suitable chemical drive the isolated inspiratory center may act as a relaxation oscillator (195) In peripheral nerve, spontaneous rhythms having a slower time course than action potential spikes are well known (8, 47, 238). The cerebral cortex deprived of all afferent connections (40) or so treated by drugs as to render synaptic transmission presumably impossible (230) may continue to show rhythmic activity Thus it is not impossible that non-propagated fluctuations in neuronal membrane potential may contribute to the EEG This in no way diminishes the significance of reverberating circuits such as those which have been postulated for cortico-thalamic (26) and cortico-hypothalamic (263) interaction Since oscillations in membrane potential tend to be associated with cyclic changes in excitability of neurones, giving rise to propagated spikes when the fluctuations are sufficiently great (47), and since the local oscillations themselves can be intensified and synchronized by arriving propagated impulses (238), it is conceivable that both the local periodicity and the total time delay of a reverberating pathway may enter into the determination of the frequency of a recorded rhythm in an active and transmitting brain

Finally, as to the functional significance of the EEG, certain generalizations must be borne in mind, particularly in evaluating drug action Although the dominant alpha rhythm is commonly considered an indicator of the resting wakeful state, it is obvious that many subjects at rest and almost all with eyes open show only a highly desynchronized and irregular low-voltage fast activity, which is also typical of most unanesthetized animals in the waking state Furthermore, it is common clinical experience that consciousness and other attributes of the functionally normal brain may persist in patients with bizarre diffuse dysrhythmias, either of pathological origin or pharmacologically induced However, there are extreme EEG states in which higher function is grossly impaired, such as the hypersynchronized hyperdischarge of the tonic-clonic and the petit mal seizure, the hypersynchrony of moderate barbiturate anesthesia, or the complete inactivity characterizing very deep anesthesia or post-seizure depression Thus the ability of the brain to receive, store and transmit information is not incompatible with a wide range of EEG patterns, except at the extremes of gross diffuse hypersynchronization, hyperactivity or complete inactivity

### III SOME HYPOTHETICAL MECHANISMS OF ACTION OF DRUGS UPON THE EEG

Having disposed of these preliminary non-pharmacological considerations of the complex origin of the EEG, one can now appropriately inquire into the known mechanisms by which drugs may alter the properties of the grossly recorded electrical activity of the brain The effects of drugs upon the properties of neurones will be the subject of a subsequent review (318), and only brief mention of some of the possible alterations will be attempted here

Among the passive properties of nerve cells, which can be measured without the elicitation of a test response, should be included the membrane resistance, capacity, inductance and voltage The capacity and inductance are not known to be easily modified by drug action, in the presence of a suitably low damping membrane resistance, they may determine the frequency of cyclic oscillations in

wide limits of convulsive bursts on the one hand or deepest anesthesia on the other. The degree of synchronization also contributes to the sharpness and complexity of wave-form, to the regularity of dominant rhythms and their stability toward sensory stimulation, etc. As to what might determine the degree of synchronization, it can only be said that there are two extreme situations resulting in synchrony. One would be any depressant effect making reverberation impossible in any but the longest available circuits. The other would be any excitant effect, such as a decrease in threshold, leading to the easy interlocking of existent circuits. The EEG alone does not offer any simple choice between these alternatives.

Another category of drug action upon the EEG includes the modification or abolition of abnormal activity, in particular the paroxysmal dysrhythmias associated with convulsive disorders. The possible mechanisms of anticonvulsant action have been reviewed elsewhere (316, 321) and will be discussed later under anticonvulsants.

If one were to attempt to compile a list of those substances whose actions upon the EEG are well described in the literature and whose mechanism of action upon neurones has also been well defined, the list would be short indeed. Furthermore, the correlation between drug effects upon properties of neurones and upon EEG manifestations would not be very impressive. For example, ether acts as a depolarizing agent upon nerve (238, 347) whereas  $\text{CO}_2$  increases membrane potential (238), yet an increase in frequency of cortical waves is the EEG manifestation usually described for both ether (136) and  $\text{CO}_2$  (126, 142, 215, 244). Some drugs which increase neuronal thresholds without depolarizing, such as the local anesthetics and DFP (17, 25, 323), may produce convulsive manifestations in the EEG (56, 57, 148), other non-depolarizing threshold-raisers such as benzimidazole (147, 317) may have relatively little effect upon the EEG even when cortical thresholds are demonstrably raised. Inconsistencies such as these illustrate the difficulties encountered in transferring our knowledge of basic neuropharmacology to an interpretation of the EEG.

In turning now to the main body of this review, namely, the description of the effects of specific substances upon the EEG, the reader should be forewarned that only in a few cases will it be possible to define a mechanism of action with any degree of satisfaction to either the pharmacologist or the neurophysiologist.

#### IV PHARMACOLOGICAL AGENTS ACTING UPON THE EEG

##### *A Substances used primarily for their central nervous effects*

1 *Predominantly depressant drugs* a Barbiturates. Because of the widespread use of barbiturates for sedation, anesthesia, and in the therapy of convulsive disorders, the EEG effects of this drug group have been studied more widely than others. Earlier reports emphasized the similarity between records obtained under barbiturate sedation and those of natural sleep (21, 136, 224). More recently there has been increasing emphasis on the initial appearance of fast activity preceding the loss of consciousness (33, 34, 64).

The intravenous administration of phenobarbital in human subjects has been

are those qualitatively new properties which result from the organization of central neurones into complex nerve nets and which give to brain functional potentialities far beyond those of the individual neurones of which it is composed. From the standpoint of the EEG, these networks provide for synchronization and rhythmicity of activity, but their physiological significance is hardly revealed by a study of the EEG. They may multiply tremendously the impulses derived from a few afferent fibers or from spontaneously firing cells, or they may channel or terminate such activity by inhibition, thereby subserving the more elementary reflex functions of brain. By suitable arrangements of re-entrant circuits they may form a self-regulating system of variable output, capable of receiving, storing and transmitting patterns of information (245). Two reflections of the organized activity of these networks are probably commonly observed in the EEG. One concerns local field effects originating in the interaction of adjacent neurones and resulting in inhibition or excitation, depending upon the polarity of the potential gradient and the rate and extent of accommodation of the responding neurones. This type of activity may be involved in the slowly spreading electrical waves which propagate through the cortical feltwork with velocity far less than that for ordinary conduction (1, 212, 255). The other concerns reverberating circuits between cortex and subcortical centers such as thalamus (26) and hypothalamus (263), which presumably contribute heavily to the stability of at least some cortical rhythms as judged by the profound disorganization of the EEG after injury to the subcortical centers. Certain theoretical properties of these circuits deserve attention. Their characteristic period may be far greater than the total conduction time around the circuit, since part of the cycle may be consumed in the building up of facilitatory activity in local internuncial pools within each center, or in the progressive inhibition of activity within these pools. Therefore drug effects upon the frequency of the observed EEG cycle might arise from any combination of actions upon the various parameters of excitation, response and recovery. That the effects of drugs upon the frequency of EEG rhythms are usually seen as sharp, qualitative transitions in period, rather as a progressive modification over a wide range, would seem to indicate the establishment of new resonant circuits when sufficient quantitative alteration in the old circuit has made resonance inefficient or impossible.

To what extent may changes in the EEG be attributed to alterations in those properties of neurones and networks already described? Of the measurable aspects of the EEG, changes in frequency are commonly reported in connection with drug action. These may reflect bona fide changes in the underlying cycles of reverberating circuits or local oscillatory behavior, as previously discussed for drugs changing the recovery phase, or they may be the spurious result of changes in degree of synchronization, with apparent fast activity resulting from the fragmentation of established rhythms on the one hand, or slow activity from the grouping of cycle fragments on the other. The voltage of the recorded discharges is likewise dependent upon the degree of synchronization, and therefore does not indicate the actual amount of neuronal activity except within the

wide limits of convulsive bursts on the one hand or deepest anesthesia on the other. The degree of synchronization also contributes to the sharpness and complexity of wave-form, to the regularity of dominant rhythms and their stability toward sensory stimulation, etc. As to what might determine the degree of synchronization, it can only be said that there are two extreme situations resulting in synchrony. One would be any depressant effect making reverberation impossible in any but the longest available circuits. The other would be any excitant effect, such as a decrease in threshold, leading to the easy interlocking of existent circuits. The EEG alone does not offer any simple choice between these alternatives.

Another category of drug action upon the EEG includes the modification or abolition of abnormal activity, in particular the paroxysmal dysrhythmias associated with convulsive disorders. The possible mechanisms of anticonvulsant action have been reviewed elsewhere (316, 321) and will be discussed later under anticonvulsants.

If one were to attempt to compile a list of those substances whose actions upon the EEG are well described in the literature and whose mechanism of action upon neurones has also been well defined, the list would be short indeed. Furthermore, the correlation between drug effects upon properties of neurones and upon EEG manifestations would not be very impressive. For example, ether acts as a depolarizing agent upon nerve (238, 347) whereas CO<sub>2</sub> increases membrane potential (238), yet an increase in frequency of cortical waves is the EEG manifestation usually described for both ether (136) and CO<sub>2</sub> (126, 142, 215, 244). Some drugs which increase neuronal thresholds without depolarizing, such as the local anesthetics and DFP (17, 25, 323), may produce convulsive manifestations in the EEG (56, 57, 148), other non-depolarizing threshold-raisers such as benzimidazole (147, 317) may have relatively little effect upon the EEG even when cortical thresholds are demonstrably raised. Inconsistencies such as these illustrate the difficulties encountered in transferring our knowledge of basic neuropharmacology to an interpretation of the EEG.

In turning now to the main body of this review, namely, the description of the effects of specific substances upon the EEG, the reader should be forewarned that only in a few cases will it be possible to define a mechanism of action with any degree of satisfaction to either the pharmacologist or the neurophysiologist.

#### IV PHARMACOLOGICAL AGENTS ACTING UPON THE EEG

##### *A Substances used primarily for their central nervous effects*

1 *Predominantly depressant drugs* a *Barbiturates* Because of the widespread use of barbiturates for sedation, anesthesia, and in the therapy of convulsive disorders, the EEG effects of this drug group have been studied more widely than others. Earlier reports emphasized the similarity between records obtained under barbiturate sedation and those of natural sleep (21, 136, 224). More recently there has been increasing emphasis on the initial appearance of fast activity preceding the loss of consciousness (33, 34, 64).

The intravenous administration of phenobarbital in human subjects has been

reported to produce either an increase in amplitude of cortical activity (224), or an initial decrease in amplitude followed by an increase as the effect progresses or when larger doses are used (141), these changes are followed in turn by a gradual slowing of the record and the appearance of normal sleep patterns. M. Lennox (216) studied the effects of relatively small doses of phenobarbital, pentobarbital, amytal and seconal in normal human subjects. Pentobarbital and seconal, in particular, produced fast activity ranging between 20 and 30 per second and most pronounced in the frontal and parietal leads. However, some subjects showed no change and some presented slow rather than fast activity. Similar variability was noted in a group of psychiatric patients receiving barbiturate medication. The observed EEG changes were not attributable to drowsiness, were more characteristic of the patient than of the drug used, and were also influenced by the dosage and the time interval before recording.

Analyses of the cortical frequency spectrum have been made by Gibbs and Maltby (141) and by Brazier (33) in an attempt to make a more quantitative evaluation of the EEG effects of barbiturates and other agents than that provided by the original EEG tracing. Gibbs and Maltby found pentothal the most effective of various barbiturates in producing slow changes, and correlated this with the greater depressant potency of the drug. Brazier routinely found the appearance of fast activity as the first manifestation of intravenously administered pentothal, and noted that during this stage there was some degree of mental clouding or euphoria. The first appearance of slow activity coincided dramatically with loss of consciousness, and fast activity disappeared at the same time. Brazier found that activity in the alpha frequency range persisted even during unconsciousness, but that it no longer could be blocked by sensory stimulation. In speculating upon the transition from the initial fast to the final slow type of record, Brazier invokes several possible alternative explanations: an initial state of acceleration by the appearance of acid metabolites, followed by the overwhelming effect of the slowing of the principal chemical reactions as a result of the accumulation of metabolites, an early depression of cortical function followed by a sharp transition to subcortical dominance, an early shift from an excitatory to an inhibitory function of the small Golgi cells (49), further transformed by the deprivation of incoming sensory impulses to the cortex. From a comparison of the effects of pentothal with those of anoxia and hypoglycemia, which produce only a progressive slowing of the EEG, Brazier concludes that the data "are consistent with a postulate that the alpha rhythm results from repetitive action of cells in neurone chains, that the rate can be modified within certain limits by metabolic changes in cortical cells, that it can be disrupted by any agent which inactivates a link in the chain, and that it is thrown out of synchrony by the arrival of action potentials originating as sensory impulses."

Regardless of the mechanism assumed, it is apparent from the foregoing studies that doses of barbiturates which are insufficient to produce sleep may produce changes in the EEG which are usually but not always in the direction of fast activity. M. Lennox (216) points out the importance of these findings in avoiding misinterpretation of the EEG of patients who are receiving barbiturate medication.

The technical difficulties encountered in attempting to obtain artifact-free recordings from infants and children have caused some electroencephalographers to use barbiturates in doses sufficient to produce sleep. Barnes *et al* (10, 11) describe 15 per second fast activity and 2 to 4 per second slow waves as typical normal EEG findings in year-old infants under light pentothal anesthesia. The reviewers (319) studied the effects of various barbiturates in a group of normal infants and children in comparison with a series of cases of convulsive disorders. In general, the sleep records were like those of normal non-sedated individuals in sleep and were dominated by slow activity of high voltage of various frequencies up to the alpha range, and variable amounts of fast activity as well. Wide variations in frequency, amplitude and regularity of the slow activity were noted. Precentral spindles of the 14 per second type were sometimes bilaterally synchronous and sometimes not, and could be evoked by sensory stimulation when they were absent from the spontaneous record at a deeper stage of sleep. There were no notable differences between the barbiturates used. The variations in EEG pattern of barbiturate-induced sleep were sufficiently wide so that the reviewers feel that such sleep records should not be considered abnormal unless they exhibit one or more of the following signs: gross asymmetry of slow activity, unilateral or bilateral absence of spindles, clearly localizable focal paroxysmal discharges, well-defined spikes, sharp waves, spike and wave patterns, or other forms normally absent from either the sleeping or waking record. Abnormal discharges should be distinguished from the high voltage K-complexes normally seen during sensory stimulation or spontaneous movement. Pentothal sleep has been found useful in the localization of convulsive dysrhythmias (116).

The EEG effects of barbiturates have also received sporadic interest in connection with their effects upon slow abnormalities frequently noted in children with behavior disorders. Cutts and Jasper (70) noted that the most striking effect of phenobarbital in such children was an increase in the amplitude of the beta activity (24-32/second), which was most marked in central and frontal bipolar recording. The effect of phenobarbital on the slower activity was to increase the amplitude and amount of 6 per second activity while decreasing that of the slower random 2 to 4 per second waves. These changes bore no correlation with the alterations in behavior in this group of children, who usually showed exacerbation of symptoms during barbiturate therapy. The addition of amphetamine to the phenobarbital medication failed to alter the EEG in these cases, although it produced clinical improvement. Lindsley and Henry (231) also found no correlation between the exacerbation of behavior disorders in children treated with phenobarbital and the alterations produced in the EEG. They report an increased amplitude of alpha rhythm in frontal leads, increased stability of precentral alpha, decreased precentral 5-8 per second slow activity with a concomitant increase occipitally and frontally.

In patients habituated to barbiturates, convulsions are sometimes noted in the several days following the withdrawal of the drug, even when the patient has no previous history of convulsive disorder. One report of such a case (51) showed hyperventilation-induced slow activity in frontal leads during the post-

withdrawal interseizure period. These were no longer evocable when more gradual withdrawal was carried out.

In summary, the clinical literature on the EEG effects of barbiturate medication shows a general but not invariable appearance of fast activity or increase of beta activity after small doses which produce minimal psychic effects, whereas larger doses sufficient to produce sleep alter the record in the direction of dominant slow rhythms with the appearance of spindles and other features of the EEG in natural sleep.

For the effects of still higher concentrations of barbiturates on the EEG, one must turn to observations on laboratory animals. Although most of the literature concerning the effects of barbiturates on the electrical activity of the brains of animals deals with pentobarbital as the test agent and the cat as the test animal, the results in general have been confirmed for other barbiturates and other species, and differ only in a few respects from the more limited range of observations in man.

Clark and Ward (60) have described somewhat more fully than earlier investigators the effects of pentobarbital on the EEG of the cat. The waking record in their animals was characterized at rest by low amplitude somewhat irregular activity in the 10-15 per second range. When the animal was alerted by sensory stimulation, an "activation pattern" of irregular low voltage fast activity in the 20-40 per second range was seen, as previously reported by Rheinberger and Jasper (280). With the onset of normal sleep, regular and high amplitude activity of 5-7 per second appeared occipitally, spindle-like formations of 14-16 per second appeared independently and asynchronously in the frontal poles, and finally random slow activity prevailed in all leads. These were essentially the changes seen with the development of light pentobarbital anesthesia. However, in contrast to normal sleep, the typical activation pattern was less easily induced, and consisted of the superposition of fast activity upon the slow rhythms, together with a lessening of the amplitude of the slow activity. At surgical levels of anesthesia the EEG was no longer alterable by painful stimulation. With still deeper anesthesia, quiescent periods appeared in the record, from which emerged bursts of slow monophasic spindles or isolated sharp waves. At still deeper levels the record became completely flat, but even after prolonged inactivity of this type the normal EEG returned when the anesthetic wore off.

Numerous experiments have been devoted to the search for a mechanism of barbiturate action upon the EEG. Bremer (37) found that various barbiturates administered to cats caused a type of cortical activity similar to that of natural sleep. Since the activity in moderate barbiturate anesthesia was similar to that seen also after transection of the brain stem, was not modifiable by sensory stimulation, and showed rhythmic activity of higher voltage than in the waking state, he concluded that barbiturates produced essentially a functional deafferentation of the cortex, permitting it to discharge in a synchronized autonomous manner. He recognized that the barbiturates must have some direct action upon the cortex itself, since there was a decreased sensitivity to the effects of locally applied strychnine.



In further analysis of barbiturate action, Bremer (39) studied responses of the auditory cortex of the cat. Normally these consist of a primary response and an afterdischarge to abrupt stimulation (handclap), and the appearance of higher frequencies at increased amplitude during continuous stimulation (whistle). Barbiturate narcosis, like natural sleep, was characterized by retention of the primary response and loss of the afterdischarge following handclap, and loss of the response to whistle. The results might be interpreted as indicating that barbiturate sedation left the cortex accessible to sensory stimuli, but abolished reverberating discharges and modifiability of cortical activity by continuous stimulation.

Drohocki and Drohocka (90) made simultaneous records from cortex and various subcortical centers in several species of animals under pentobarbital or amytal anesthesia. They were impressed by the qualitative change from low voltage fast activity of the waking state to high voltage slow waves having many new properties unlike those of normal activity. Since these qualitative changes occurred simultaneously in cortex and thalamus, they concluded that there was no specific locus of action of the barbiturates.

Hembecker and Bartley (178) attempted to define the mechanism of action of pentobarbital by studies at various levels of the nervous system. They found that responses in the sensorimotor cortex of the rabbit evoked by stimulation of the saphenous nerve were depressed even in light pentobarbital anesthesia before notable changes occurred in the spontaneous cortical activity. The late components of the evoked response attributable to stimulation of C fibers of the saphenous nerve were the first to be depressed, this suggests an electrical counterpart of the early obtundation of pain under light anesthesia which leaves other sensory modalities intact. The period of facilitation normally seen after local stimulation of the cortex was delayed and reduced in extent. In the spinal cord of the turtle, reflex motor discharges elicited by stimulation of sensory trunks were regularly diminished in amplitude and duration, with only occasional evidence of a transient initial excitatory effect. Temporal summation and later facilitation were reduced in the excised superior cervical ganglion of the turtle. The small unmyelinated fibers of the vagus in the turtle were the first to be blocked by pentobarbital, while the large myelinated fibers were the most resistant. Spontaneous discharges from the ganglion cells of the limulus heart were slowed by pentobarbital. A variety of effects were noted after the application of pentobarbital to frog sciatic nerve, including reduction in amplitude of spike and negative afterpotential, increased threshold, prolongation of absolute and relative refractory period, slowing of conduction velocity, and a slight decrease in extent of accommodation. Since the concentrations used were inordinately high in all but the cortical observations, there might be some question concerning the relevance of these findings to the action of the barbiturate upon the brain. However, the peripheral nerve data are compatible with the effects observed upon the cortex in that no excitatory effects were seen, thresholds were increased, recovery time delayed, facilitation reduced, and spontaneous rhythms slowed.

A number of investigators have attempted to define the centers and anatomical pathways involved in the elaboration of the several types of electrical activity recorded from the cortex during the course of barbiturate action. The simplest of these types would appear to be the highly localized, brief, monophasic, surface-positive response to sensory stimulation which represents the invasion of the cortex by afferent impulses. This response has been widely used in mapping the representation of body surface and special sense organs upon the cortex. Marshall, Woolsey, and Bard (244), in the course of their topographical studies, noted that pentobarbital did not affect any of the characteristics of this primary response except for its recovery time, which was greatly prolonged. Since the primary response can be observed at any level of anesthesia and in the unanesthetized animal, it undoubtedly contributes to the recorded "spontaneous" activity as long as any sensory impulses are being propagated along the ascending tracts. However, under deep pentobarbital anesthesia a mechanism has been described by Marshall and his collaborators which operates to limit the amount of sensory activity reaching the cortex. Marshall *et al* (243, 244) observed the effects of pentobarbital on responses evoked by discrete tactile stimulation of the hair of a forefoot of the cat. At the level of the cortex, there was a prolongation of the recovery time (cf 198) and little other change in the character of the highly localized primary response, a reduction in complexity of various secondary reactions, and the loss of long-lasting facilitation effects. Marshall (243) found that the absolute unresponsive time was prolonged at the thalamic level, rostral to the ventrolateral nucleus pars externa, but not caudally, so that the normally arriving continuous barrage of lemniscus impulses tended to produce only grouped discharges after transmission through the thalamus.

In addition to the primary responses, sensory stimulation may set off several types of more generalized and drug-sensitive secondary discharges (243, 244). One of these has been described in more detail by Forbes and Morison (113) who studied the responses evoked in the cortex of the cat by sciatic nerve stimulation under conditions of relatively deep pentobarbital or dial anesthesia, when spontaneous activity was absent or consisted only of occasional slow bursts. The evoked responses were characterized by brief and relatively local primary responses which continued undiminished with fairly rapid repetitive stimulation, and by more prolonged and generalized secondary discharges resembling the spontaneous bursts, which were characterized by easy fatigability during rapid stimulation and a refractory period of a second or more. Thus it would appear that the relatively quiescent cortex under deep barbiturate anesthesia is not only still accessible to sensory impulses, but that these may set off generalized secondary discharges of slow wave type, such discharges may also arise spontaneously in the absence of apparent stimulation.

A further analysis of these secondary discharges has been made by Swank *et al* (310, 311, 312) who investigated the effects of amytal on cortical activity of the dog. In the normal resting state these animals typically showed dominant 25 per second activity, mixed with 12 per second activity which was more prominent posteriorly, and 50 per second activity anteriorly, the latter predominating

in states of enhanced excitement. During the induction stage of amytal narcosis, the faster activity disappeared first, while spindle activity occurred. In deeper narcosis there was a progressive slowing of dominant frequency, with least change in the motor area. In deep surgical anesthesia the record was characterized by alternating slow bursts and periods of inactivity, these periods of suppression occurring first in the occipital cortex. The suppression phenomenon could be partly reversed by administration of oxygen. The synchronized bursts of activity occurring at this level of anesthesia were shown to originate independently in either hemisphere or in the lateral nuclei of either thalamus, and to be rapidly propagated through both hemispheres in a few milliseconds. Propagation between the hemispheres occurred by way of the corpus callosum, and within the hemispheres by way of the internal capsule and lateral portion of the thalamus. The medial nuclei and massa intermedia of the thalamus were not involved, although these may play an important role in cortical synchronization in lighter anesthesia (200). At this deep level of anesthesia fast activity was still recorded independently in the pons and reticular formation of the midbrain. Cortical thresholds for evoked discharges were considerably raised at this stage and were particularly high at the end of each isolated burst, recovering slowly over a period of several seconds. Evoked afterdischarge could not be elicited even under light anesthesia.

Swank *et al.* attempted to explain their findings on the basis that barbiturates first depress the function of the smaller neurones, leaving only activity which can originate and propagate in the largest cells, on the basis of the finding of Heinbecker and Bartley (178) that barbiturates first depressed small fibers in peripheral nerve and cord tracts. However, their results illustrate directly two other important actions of barbiturates, namely, increase in threshold and prolongation of recovery time.

These observations suggest the existence even in deep barbiturate anesthesia of a potential reverberating circuit involving cortex and lateral thalamic nuclei and capable of synchronizing a large mass of brain tissue. That the circuit fires only sporadically in deep anesthesia is easily explained by the slow recovery time. In lighter narcosis this limiting factor disappears, and high voltage slow activity now dominates the EEG.

As an alternative mechanism, the reviewers would like to suggest that the sporadically firing mechanism seen in deep narcosis becomes more highly fractionated as the recovery time is shortened toward normal, this results in the appearance of more asynchronous and superficially faster activity. In studies of evoked cortical responses in the rabbit (315), the reviewers have followed the progress of the slow secondary component at all stages from deep barbiturate anesthesia to metrazol convulsions. Even in the resting untreated animal the slow component fires only once, and only with subconvulsive doses of metrazol does it develop the rhythmic character expected of a reverberating circuit. Therefore, it seems plausible to postulate that the action of barbiturates in slowing the grossly recorded EEG is neither the prolongation of the transit time around a fixed circuit, nor the slowing of an intrinsic frequency of a sinusoidal

oscillator, but the obligatory condensation of normally fractionated and asynchronous neurone populations by virtue of the delay in their recovery times

As to the origin of the spindle type of activity appearing in the EEG of the sensorimotor cortex under barbiturate sedation, Morison *et al* (255, 256) have shown that it continues to occur in the thalamus, unilaterally or bilaterally, after bilateral removal of the cerebral cortex, which indicates that reverberating circuits through the cortex are not essential. Obrador (263) has shown that the discharges are abolished by lesions limited to the hypothalamus and basal regions of the brain, and by lesions of the thalamus and thalamocortical pathways, but not by section of the brain stem (cf 37). Hoagland *et al* (189) have shown that the hypothalamus is more resistant than the cortex to the slowing effects of pentobarbital. From these observations it would appear that the sensorimotor cortex passively received the spindle bursts from a more rugged system of thalamic and hypothalamic nuclei. This system may constitute a true reverberating circuit, since trains of cortical spindle activity rather than isolated spikes are elicited by single shocks delivered to the cortex in light barbiturate anesthesia or even in the waking state (315). Why they should be relatively limited to the sensorimotor cortex and why they should be characteristic of only a limited range of sleep or anesthesia are unanswered questions.

An even more difficult problem concerns the origin of the generalized fast activity in light barbiturate anesthesia, particularly in man. It may represent intrinsic cortical activity, since Bishop (26) and Chatfield and Dempsey (53) have found that frequencies faster than the alpha range persist after section of the thalamic radiations. Activity even in the alpha range need not depend on subcortical reverberations, but may result from periodic summation in the cortex subjected to a constant subcortical bombardment of subliminal impulses, according to the studies of Dempsey and Morison (85, 86). Kristiansen and Courtois (210) have recently shown that even isolated portions of cerebral cortex continue to show 8-12/second discharges. That at least the upper layers of cortex may behave relatively independently is indicated in studies of their electrical activity and excitability by Adrian (1) and Rosenblueth and Cannon (285). However, it should be noted that thermocoagulation of the outer three layers does not abolish cortical activity in the alpha range of frequencies, according to Dusser de Barenne and McCulloch (91). Pending a more precise localization of alpha and faster activity, it is not impossible that the fast waves of light barbiturate anesthesia are of cortical origin and represent some degree of autorhythmicity.

A quite different mechanism for the initially observed changes in light barbiturate anesthesia might be the progressive synchronization of afferent discharges by a slowing of the recovery time of the thalamus, as shown by Marshall (243), coupled with similar slowing in the cortex. This effect would tend to cause the appearance of recordable fast discharges from a previously completely asynchronous and therefore unrecorded background of arriving sensory impulses. Whatever the anatomical localization of the faster activity may be, it cannot represent a fundamental change in cortical function, since it may occur in the

presence of little or no impairment of consciousness, as previously noted. The transformation of this state into the qualitatively different electrical and psychic activity of sleep constitutes one of the most dramatic and challenging problems in neurophysiology.

In the entire foregoing description, there seems to be nothing incompatible with the view that the essential mechanisms of barbiturate action are increased recovery time and increased threshold for cerebral neurones in general, with a somewhat greater sensitivity of cortex than of diencephalic centers. In this light the various qualitative changes in the EEG should be looked upon not as the sudden appearance of an additional type of drug action, but as an expression of the functional organization of the brain itself, which can operate within several semistable states of self-regulation, the state being determined in part by the prevailing time and voltage parameters of excitability, in part by the inflow of afferent impulses, and in part by some as yet unknown perversities that characterize a machine evolved for learning and dreaming.

b General anesthetics. Despite the wide use of general anesthetics, EEG studies have been relatively few. In one of the first demonstrations of electrical activity in the brain of animals, Fleischl von Marxow (110) reported in 1890 that the electrical activity of the cerebral cortex could be modified by chloroform. In the first equivalent observations on the human EEG, Berger (21) observed that the alpha waves in man were greatly increased in amplitude during the excitement phase of chloroform anesthesia, but that alpha activity was abolished in surgical anesthesia with chloroform.

The effects of ethyl ether have been studied in slightly more detail. Gibbs, Gibbs and Lennox (136) noted in 1937 that during the early stages of ether anesthesia there was first an increase in voltage in frequencies around 20 per second, with a diminution in voltage in frequencies around 10 per second. As consciousness was lost these high voltage fast waves disappeared, giving way to high voltage slow waves at the rate of about 5 per second. The latter gradually were reduced to a frequency of 1 per second, with some persistent 10 to 20 per second activity superimposed, as surgical anesthesia was reached.

In contrast to ether, induction with nitrous oxide does not appear to produce a pattern of fast activity. Derbyshire *et al* (87) observed that the number of alpha waves per unit time was decreased with mixtures up to 70 per cent nitrous oxide, first in the motor areas and subsequently in the occipital region. Mixtures of 70 to 90 per cent nitrous oxide produced slowing of the pattern to 4-8 per second in most subjects, particularly evident in the motor area. With pure nitrous oxide two possible patterns appeared. A frequency of 2.5 per second with an amplitude of 250 microvolts occurred if the induction was rapid, but if the patient had a slower induction followed by pure nitrous oxide, an irregular delta pattern of about 40 microvolts was evoked. Both patterns appeared simultaneously with beginning cyanosis of the nail bed. Wide individual variations of the EEG pattern were encountered for each particular mixture of nitrous oxide and oxygen. In contrast, mixtures of nitrogen and oxygen produced no change in normal subjects when the oxygen content was above 10 per cent, however,

normal subjects exposed for a short time to very low oxygen tensions showed an increase in voltage and the appearance of slow 4-8 per second rhythms in the EEG. Although the effects of nitrous oxide somewhat resemble those of anoxia, they are not simply the result of the relative anoxia frequently encountered during the use of this anesthetic agent.

The effects of cyclopropane on brain potentials in man were studied by Rubin and Freeman (291, 292). Initially, after a mixture of cyclopropane (350 cc) and oxygen (250 cc per minute) was breathed for one to two minutes, there was noted a decrease in frequency to 7-8 per second with an increase in amplitude of 100 per cent or more. At this stage the patient was in light anesthesia, with disappearance of the corneal reflexes. The next stage was marked by an increase in amplitude, with the appearance of regular 3 per second waves at the onset of surgical anesthesia. Electrocorical changes during recovery did not follow those observed during induction. If the subject was not kept in deep anesthesia more than 3 to 5 minutes, the early stages of recovery were extremely rapid. When the patient was allowed to breathe warm air, low voltage waves of mixed frequency appeared immediately. However, if the period of surgical anesthesia had been appreciably longer than 5 minutes, upon substitution of warm air for the cyclopropane-oxygen mixture there was further slowing of frequency to less than 3 per second, this was attributed by the authors to reflex vasodilatation and consequent flooding of the brain with increased amounts of cyclopropane. The next stage of recovery was characterized by an increase in frequency to a predominate pattern of 18 per second with a decrease in amplitude, in some instances this stage was preceded by a regular 7-8 per second rhythm. The final stage was marked by a decrease in frequencies to 12-14 per second, with eventual restoration of the normal waking pattern. The amplitude and regularity of slow potential changes during anesthesia were greatest in the frontal lobe, and were less regular and of lower amplitude posteriorly. The 12-14 per second activity during recovery was most marked in the motor and pre-motor areas, where the waking potential pattern was first established after discontinuance of cyclopropane. It is certainly not clear to what extent the changes during recovery represent direct effects of the anesthetic as opposed to those of post-anesthetic sleep. More detailed analysis of the induction period is desirable.

Laboratory observations with regard to the influence of general anesthetics on cortical potentials have been rather more extensive than clinical reports. Beecher and McDonough (13, 14) studied in detail the effects of 17 anesthetics on cortical action potentials from the sensory areas in cats. Two levels of anesthesia were arbitrarily chosen: the lightest anesthesia which could be maintained without producing generalized muscular response on sciatic stimulation, and the level at which the flexion reflex just disappeared. They were able to classify the anesthetics into two distinct groups. The first consisted of those which were volatile (exception for urethane) and of low molecular weight, these produced a dominant frequency under light anesthesia of more than 26 per second. The second consisted of non-volatile anesthetics, including tribromoethanol (avertin), evipal, paraldehyde, barbital, chloralose and pentobarbital, these produced

high voltage 1-10 per second waves on which faster activity of low voltage were superimposed. The high voltage slow waves appeared in bursts at 3 to 5 second intervals, each lasting 1 to 2 seconds. Anesthesia with the non-volatile agents more nearly resembled natural sleep than did that induced by the volatile agents. Central sciatic stimulation failed to alter cortical discharges under light or deep anesthesia with any of the non-volatile anesthetics, but under light anesthesia with the volatile group, such stimulation greatly increased the voltage of the cortical waves.

Beecher (13) reported similar studies on a series of alcohols, all of which behaved like the volatile anesthetics with respect to their effect on electrical cortical activity. The pattern during light anesthesia was characterized by rapid small amplitude waves, and an increase in voltage was elicited by sciatic stimulation. The average frequency of the waves was inversely related to the anesthetic potency which in turn was directly related to the molecular weight, except that the secondary and tertiary alcohols were more potent than the primary alcohols.

The effects of ether on the EEG of the dog have been analyzed by Swank and Watson (312). They found that the fastest activity (50 per second) in the normal EEG was at first increased in amplitude by ether, beginning in the parietal area and then in the premotor area, while it was decreased in the motor area. In deep anesthesia, low amplitude slow waves began to replace the fast activity concomitant with the disappearance of the corneal reflex. Bursts of spindle activity to that seen during light barbiturate anesthesia occurred during recovery phase, but since these were also observed in the hemispheres of decerebrate animals which had fully recovered from ether anesthesia (37) and are a characteristic of the normal sleep record, the spindles should probably be attributed to post-anesthetic sleep rather than to the effect of ether itself. During the induction and recovery stages of ether anesthesia, cortical thresholds for the elicitation of afterdischarge (i.e., seizure activity) were lower than normal, particularly in the motor cortex, but at surgical levels of anesthesia, afterdischarge could not be obtained.

Forbes *et al.* (112) observed mixed fast and slow activity in light ether anesthesia in the EEG of the cat, reverting to slow activity as the anesthetic was deepened, in contrast to the spindle and high voltage slow sequence of pentobarbital or tribromoethanol. They were impressed by the parallelism between potentials evoked by sciatic stimulation and the spontaneous cortical activity at various levels of depression by these three drugs, and concluded that the anesthetics acted not merely by blocking afferent impulses but by directly modifying the action of the cortex.

An interesting contrast between ether and barbiturates has been reported by Marshall, Woolsey and Bard (244) who noted that ether did not alter the threshold, latency or size of the primary response evoked in the somesthetic area of the cortex of the cat by tactile stimulation of the body surface. Even with deep ether anesthesia the recovery time was shorter than with pentobarbital, and with moderately light ether it was relatively normal.

Possibly related to this difference between ether and barbiturates are some

observations of Bremer (39), who found that ether anesthesia depressed equally the primary and secondary responses of the auditory cortex of the cat to abrupt auditory stimulus, without altering the fast activity which normally appeared in response to a continuous whistle. In contrast, the barbiturates abolished the whistle and secondary responses in doses which did not impair the primary response. With regard to the primary response, it should be noted that Marshall, Woolsey and Bard (244) did not find any reduction of amplitude under ether anesthesia, but found the action potential harder to identify and localize because of the background fast activity. The differential effects of ether and barbiturates on the whistle response would seem to indicate that ether does not impair the ability of the cortex to respond to stimulation of high frequency to the same extent as do the barbiturates.

Still another difference between ether and barbiturates has been reported by Forbes and Morison (113). They observed that ether added to preexisting deep barbiturate anesthesia, up to the point of respiratory failure, abolished the secondary cortical responses to peripheral nerve stimulation without altering the primary response. Deepening the barbiturate narcosis alone did not abolish the secondary responses. These investigators had previously shown that the secondary discharges were not characteristic of deep ether anesthesia. The implications of their studies are two-fold: first, that afferent impulses may still reach the cortex at levels of ether or barbiturate anesthesia sufficient to abolish any spontaneous activity of the cortex; secondly, that while the barbiturates may leave the cortex in a quiescent state from which secondary discharges may still be elicited, ether has a more profound effect on the ability of the cortex to respond at all.

In an attempt to differentiate the mechanisms of action of ether and barbiturates on a more simple reflex system, Beecher *et al* (15) compared the effects of evipal and ether on flexion reflexes mediated through the spinal cord of the cat. Ether did not prevent the development of sustained and cumulative responses to rapid reflex stimulation, in contrast to evipal which permitted only discrete responses to individual stimuli. Likewise they found that afterdischarge following the cessation of stimulation was more seriously impaired under barbiturate anesthesia than under ether, and concluded that "long-circuiting" of sensory impulses along internuncial chains was less curtailed by ether. This explanation is of course more descriptive than definitive, since the possible actions leading to failure of internuncial reverberation are not taken into account. One of these actions, prolongation of recovery time, has been a universal finding with barbiturates but not with ether.

The systematic investigations of Hembecker and Bartley (178) on the mechanism of action of pentobarbital have already been discussed. Parallel observations on the effects of ether showed many similarities at all levels of the nervous system, but a few differences are worthy of mention. Ether increased while pentobarbital slightly decreased the extent of accommodation in frog sciatic nerve. The rhythmic discharge of impulses from neurones of the *Limulus* heart ganglion was typically accelerated by ether and slowed by pentobarbital. Reflex dis-



charges initiated from the spinal cord of the turtle were initially increased in frequency and duration by ether, whereas pentobarbital usually had a progressive depressant effect without initial excitation. Ether increased whereas pentobarbital decreased the frequency of impulses in the phrenic nerve associated with respiration. Ether increased the frequency of cortical spontaneous activity in the rabbit, in contrast to the sleep pattern seen after pentobarbital, and deeper ether anesthesia reduced the amplitude of the EEG progressively, the effect occurring earlier in cortex than in thalamus. The secondary period of facilitation following a cortical response to saphenous nerve stimulation occurred earlier than normal with ether and was delayed by pentobarbital, although both drugs reduced the magnitude of the facilitation effect. This latter phenomenon would seem to bear a more direct relation to the increase in cortical frequencies than to some of the other effects studied by Heinbecker and Bartley. At any rate it is of interest to note that increased frequency of electrical rhythms and some evidence of an initial excitatory effect of ether have been seen in other tissues than brain.

As to the basic mechanism of action of ether, Lorente de N6 (238) found that conduction block produced by ether in frog sciatic nerve was attributable to depolarization, but that the excitability of the blocked fibers could be restored if the membrane potential was artificially increased toward normal by an applied anodal current. In the earliest stage of ether action there was a tendency toward increased excitability associated with an increase in membrane potential before the ultimate depolarizing effect set in. That the excitability may eventually be progressively diminished by increasing the ether concentration is suggested by the work of Wright (347) who found that the exposure of mammalian peripheral nerves to ether vapor resulted in a progressive depolarization, loss of action potential amplitude, and increase in threshold. The last named effect was unlike that of anoxia, which was found to have little effect on threshold until critical depolarization causing conduction failure was achieved, at which point the threshold rose precipitously. Ether in high concentrations blocked conduction with relatively little change in membrane potential. The effects of ether seemed to be independent of the presence or absence of oxygen.

If ether acts primarily as a depolarizing agent, it might also be expected to increase membrane permeability if the mechanism of action were similar to that of potassium (193). However, Spiegel and Spiegel-Adolf (302) have concluded from conductivity measurements that the permeability of brain cells is decreased by ether and chloroform as well as by the barbiturate Dial. Whether such measurements truly reflect changes in cell permeability might be questioned, since decreased neural activity itself, secondary to the anesthesia, might reduce the average permeability of brain cells by reducing the number of impulses per unit time and therefore the number of occasions in which the membrane resistance is transiently reduced. It is hoped that in the future a large body of valid observations on the effects of drugs on resting membrane resistance will be forthcoming, since the concept of change in permeability has all too frequently been invoked without rigorous experimental support.

observations of Bremer (39), who found that ether anesthesia depressed equally the primary and secondary responses of the auditory cortex of the cat to abrupt auditory stimulus, without altering the fast activity which normally appeared in response to a continuous whistle. In contrast, the barbiturates abolished the whistle and secondary responses in doses which did not impair the primary response. With regard to the primary response, it should be noted that Marshall, Woolsey and Bard (244) did not find any reduction of amplitude under ether anesthesia, but found the action potential harder to identify and localize because of the background fast activity. The differential effects of ether and barbiturates on the whistle response would seem to indicate that ether does not impair the ability of the cortex to respond to stimulation of high frequency to the same extent as do the barbiturates.

Still another difference between ether and barbiturates has been reported by Forbes and Morison (113). They observed that ether added to preexisting deep barbiturate anesthesia, up to the point of respiratory failure, abolished the secondary cortical responses to peripheral nerve stimulation without altering the primary response. Deepening the barbiturate narcosis alone did not abolish the secondary responses. These investigators had previously shown that the secondary discharges were not characteristic of deep ether anesthesia. The implications of their studies are two-fold: first, that afferent impulses may still reach the cortex at levels of ether or barbiturate anesthesia sufficient to abolish any spontaneous activity of the cortex, secondly, that while the barbiturates may leave the cortex in a quiescent state from which secondary discharges may still be elicited, ether has a more profound effect on the ability of the cortex to respond at all.

In an attempt to differentiate the mechanisms of action of ether and barbiturates on a more simple reflex system, Beecher *et al* (15) compared the effects of evipal and ether on flexion reflexes mediated through the spinal cord of the cat. Ether did not prevent the development of sustained and cumulative responses to rapid reflex stimulation, in contrast to evipal which permitted only discrete responses to individual stimuli. Likewise they found that afterdischarge following the cessation of stimulation was more seriously impaired under barbiturate anesthesia than under ether, and concluded that "long-circuiting" of sensory impulses along internuncial chains was less curtailed by ether. This explanation is of course more descriptive than definitive, since the possible actions leading to failure of internuncial reverberation are not taken into account. One of these actions, prolongation of recovery time, has been a universal finding with barbiturates but not with ether.

The systematic investigations of Heinbecker and Bartley (178) on the mechanism of action of pentobarbital have already been discussed. Parallel observations on the effects of ether showed many similarities at all levels of the nervous system, but a few differences are worthy of mention. Ether increased while pentobarbital slightly decreased the extent of accommodation in frog sciatic nerve. The rhythmic discharge of impulses from neurones of the *Limulus* heart ganglion was typically accelerated by ether and slowed by pentobarbital. Reflex dis-

of statistical significance. Other subjects with no previous history of addiction were given single doses of 12 to 15 mgm, sufficient to produce marked symptoms consisting of nausea, vomiting, lassitude and general depression without preliminary excitement. Two cases showed no EEG changes, and the third exhibited a typical sleep pattern three to four hours after injection, although the patient was definitely not asleep during recording. During this period the alpha rhythm could be brought back temporarily by repeated light or sound stimuli.

Andrews (7) also observed the effect of meperidine in five patients who were previously addicted to opiates but had received no drug for at least six months. An initial dose of 100 mgm daily was given, and each subject then chose his own frequency and dose within imposed limits of  $1\frac{1}{2}$  hours and 300 mgm. The EEG was recorded each week throughout the study period and every fifteen days after withdrawal until the record returned to the pre-experimental norm. Early in the study, the EEG showed slow waves which became progressively slower and of greater amplitude. After withdrawal, the slow waves persisted for about 48 hours, after which there was a progressive return to the original type of record. When slow wave activity occurred in response to morphine, it was relatively slower but also more transient than that seen after meperidine.

The effects of methadone have been studied by Isbell *et al* (197). Single doses of less than 30 mgm failed to produce significant changes in the EEG of normal subjects. However, one subject showed a marked slowing of frequencies following 30 mgm of methadone. No definite correlation could be made between the EEG changes and the sedation produced by the drug, but the degree of sedation was difficult to measure. Similar EEG changes were seen in all patients receiving repeated smaller doses.

In contrast to these observations in man, Leimdorfer (214) has noted a tendency toward fast activity and spiking in the EEG of cats given either morphine or meperidine subcutaneously. It should be recalled that in the cat the effects of morphine are predominantly excitatory, with frequent occurrence of frank seizures.

Because of species differences in response to analgesics, it is difficult to relate the results of animal experimentation to the changes noted in the EEG of man. Wikler (338, 339, 340, 341) has made a careful study of the action of morphine at all levels of the central nervous system. He found that morphine in relatively small doses enhanced two-neurone reflexes while depressing multineuronal responses in the spinal cord of the cat. In contrast, pentobarbital and ether depressed both types of response. Eserine enhanced both types of discharge following a small dose of morphine, but had no effect on the multineuronal reflexes when given alone. This finding is of some interest in view of a possible cholinergic action of morphine. Observations of the mixed signs of excitation and depression produced by morphine at various levels of the cerebrospinal axis led Wikler to conclude that this alkaloid produced selective depression of interneurons accompanied by release phenomena, and to suggest that species differences may pertain to differences in neural organization. Until further evidence is forthcoming, it would seem wise to withhold judgment on the mechanism of action of

From the foregoing observations, it can be seen that among the general anesthetics only ether has been analyzed sufficiently to permit some tentative conclusions concerning mechanism of action. The ultimate action of ether upon neurones is depolarization, but it is doubtful whether a depth of anesthesia sufficient to produce conduction failure by depolarization throughout the nervous system is ever reached. It is not unlikely that a moderate degree of depolarization is critical for synaptic transmission in the cortex and occurs at lighter levels of anesthesia. At any rate it may be stated with assurance that prolongation of recovery time is not an important factor in ether anesthesia.

The following picture of the effect of ether anesthesia on the EEG may now be synthesized. Under light anesthesia, the frequency of spontaneous activity may be increased, concomitant with a shortening of the facilitation interval and in accord with the action of ether on other nervous aggregates. In spite of this effect and overriding a moderate increase in excitability there may be a progressive failure of synaptic transmission within the cortex as the most sensitive synaptic regions partially depolarize. Slow activity of low amplitude and probable subcortical origin may appear, but not the high voltage discharges characteristic of barbiturate anesthesia, the subcortical reverberations represented by spindles do not occur. Eventually the cortex and related structures may become unresponsive in deepest ether anesthesia, but at this stage the afferent systems are still able to project to the cortex. By more refined analysis, particularly of the extent of the depolarization effect, it should be possible to verify or reject this tentative conception and to evaluate the role of threshold changes induced by ether.

c Analgesics. The EEG effects of analgesics have generally been described as slowing of frequency or the appearance of sleep-like records. Thus, according to Gibbs *et al* (136, 141), the intravenous administration of a single dose of morphine in normal subjects produces changes similar to those observed during normal sleep or following the administration of phenobarbital or pentothal. Patients receiving morphine were found by Peterson *et al* (269) to have more slow wave EEG abnormalities than untreated patients in response to the same degree of anoxia at high altitudes.

The most extensive studies on the effects of morphine in addicted patients have been conducted by Andrews (6). His results show interesting differences in response to morphine, apparently dependent on the prior status of addiction. For example, he studied a group of patients with previously well-established physical dependence on morphine. In this group, following the withdrawal of all opiates and the appearance of the characteristic withdrawal syndrome, the administration of sufficient morphine to prevent physiological signs of abstinence gave an abnormally high percent time alpha. Results were somewhat different in a group of post-addicts who had received no narcotics for at least one year. In no case was there any significant change in the brain potential rhythms following injection of a single dose of 20 mgm of morphine sulphate, sufficient to produce mild stimulation. However, there was a tendency for the occipital alpha blocking time to increase following injection, an incidence considered to be on the borderline

of statistical significance. Other subjects with no previous history of addiction were given single doses of 12 to 15 mgm., sufficient to produce marked symptoms consisting of nausea, vomiting, lassitude and general depression without preliminary excitement. Two cases showed no EEG changes, and the third exhibited a typical sleep pattern three to four hours after injection, although the patient was definitely not asleep during recording. During this period the alpha rhythm could be brought back temporarily by repeated light or sound stimuli.

Andrews (7) also observed the effect of meperidine in five patients who were previously addicted to opiates but had received no drug for at least six months. An initial dose of 100 mgm. daily was given, and each subject then chose his own frequency and dose within imposed limits of  $1\frac{1}{2}$  hours and 300 mgm. The EEG was recorded each week throughout the study period and every fifteen days after withdrawal until the record returned to the pre-experimental norm. Early in the study, the EEG showed slow waves which became progressively slower and of greater amplitude. After withdrawal, the slow waves persisted for about 48 hours, after which there was a progressive return to the original type of record. When slow wave activity occurred in response to morphine, it was relatively slower but also more transient than that seen after meperidine.

The effects of methadone have been studied by Isbell *et al* (197). Single doses of less than 30 mgm. failed to produce significant changes in the EEG of normal subjects. However, one subject showed a marked slowing of frequencies following 30 mgm. of methadone. No definite correlation could be made between the EEG changes and the sedation produced by the drug, but the degree of sedation was difficult to measure. Similar EEG changes were seen in all patients receiving repeated smaller doses.

In contrast to these observations in man, Leimdorfer (214) has noted a tendency toward fast activity and spiking in the EEG of cats given either morphine or meperidine subcutaneously. It should be recalled that in the cat the effects of morphine are predominantly excitatory, with frequent occurrence of frank seizures.

Because of species differences in response to analgesics, it is difficult to relate the results of animal experimentation to the changes noted in the EEG of man. Wikler (338, 339, 340, 341) has made a careful study of the action of morphine at all levels of the central nervous system. He found that morphine in relatively small doses enhanced two-neurone reflexes while depressing multineuronal responses in the spinal cord of the cat. In contrast, pentobarbital and ether depressed both types of response. Eserine enhanced both types of discharge following a small dose of morphine, but had no effect on the multineuronal reflexes when given alone. This finding is of some interest in view of a possible cholinergic action of morphine. Observations of the mixed signs of excitation and depression produced by morphine at various levels of the cerebrospinal axis led Wikler to conclude that this alkaloid produced selective depression of interneurons accompanied by release phenomena, and to suggest that species differences may pertain to differences in neural organization. Until further evidence is forthcoming, it would seem wise to withhold judgment on the mechanism of action of

morphine and other analgesics on the EEG, and the possible relationship of those effects to analgesia

d Alcohol Reports on the effects of ethyl alcohol upon the EEG have usually described a slowing of activity Thus Loomis, Harvey and Hobart (237) noted an increase in amplitude of brain potentials and slowing of the rhythm during alcoholic stupor Engel *et al* (99), in a study of normal subjects and chronic alcoholics during acute intoxication, observed progressive slowing of the brain waves accompanying the development of intoxication The degree of slowing produced a more reliable index of intoxication than the development of any particular frequencies The mean frequency of the EEG was decreased by two to three cycles per second in association with intoxication Patients who had abnormally fast records showed more normal discharges during intoxication With recovery the EEGs returned to the pre-intoxication status Very close correlation was demonstrated between the EEG and the level of consciousness, but no correlation was evident with various aspects of behavior Greenblatt *et al* (161) reported an incidence of 24 per cent abnormal EEGs among 157 patients with chronic alcoholism, alcoholic psychoses and alcoholic convulsions An increasing incidence of dysrhythmias was found when the various chronic alcoholic disorders were roughly classified according to the severity of the clinical picture, the chronicity of symptoms, and the assumed severity and extent of damage to the brain Seventeen per cent of those with "rum fits" or seizures associated with alcoholism had abnormal records, a lower incidence than for the group as a whole

Somewhat related observations were made by Kaufman *et al* (204) who administered 40 to 150 cc of 10 per cent alcohol intravenously over a period of 2 to 3 minutes to 16 patients All individuals examined showed clinical evidence of alcoholic intoxication In four instances the alcohol level reached 100 to 150 mgm per cent No alterations in the EEG patterns were observed in four non-epileptics The epileptic group showed increasing prominence of the alpha rhythm, and previously abnormal activity decreased That the rate of development of alcoholic intoxication is a factor in the relative preponderance of EEG over clinical signs is suggested by a comparison of these results with those of Davis *et al* (83) who studied the effects of alcohol on the EEG and on psychometric performance in six normal male subjects Using a Grass analyzer for quantitation of the frequency components of the EEG, they found that low concentrations of alcohol reduced activity particularly in the range of 10 to 13 cycles per second With higher concentrations, episodes of slow waves of 4-8 per second appeared in the frequency spectrum There was a definite impairment of the subjects' performance in psychometric tests at the higher blood concentrations of alcohol (125 to 140 mgm %), but the EEG continued to show abnormalities for some time after recovery of intellectual functions

In regard to possible mechanisms of action of alcohol, Wright (347) found that exposure of mammalian peripheral nerves to alcohol vapor resulted in a concomitant reduction in membrane potential and action potential amplitude, associated with a progressive rise in threshold The last-named effect was in

contrast to that of anoxia, in which condition the threshold was found to remain relatively constant until a critical value of depolarization was reached and conduction failure supervened, at which point the threshold rose precipitously

Thus, although alcohol produces a progressive slowing of the EEG not unlike that of anoxia, it should not be concluded that the mechanisms are identical. Further research upon the brain itself would be required to determine the relative importance of the threshold-raising and depolarizing effects of ethyl alcohol, and it is not impossible that still other changes as yet uninvestigated may play a role in the progressive slowing of cortical frequencies

e Anticonvulsants It has now become established with fair certainty that drugs used in the treatment of convulsive disorders need not have a depressant action upon the central nervous system (321), and therefore it is not surprising that there are no universal EEG findings during anticonvulsant medication equivalent to those seen, for example, with adequate doses of the various sedatives

Two distinct types of EEG change should be taken into account in any discussion of the anticonvulsants. The first is the alteration in the cortical electrical activity of normal subjects, or in normal interseizure records of patients of abnormal EEG manifestations. These two categories seem to involve mechanisms sufficiently different to merit separate consideration

*Bromide*, the oldest of the effective antiepileptic agents, might be expected to produce EEG alterations when the concentration in body fluids is sufficient to produce psychic disturbances. Since the therapeutic margin of safety is relatively narrow in the treatment of epilepsy with bromide, and because of the wide and unregulated use of this anion, the central manifestations of bromide intoxication are frequently encountered. In a series of cases observed by Greenblatt *et al* (162), 18 per cent of patients showed some EEG abnormalities with serum bromide concentrations of 100 mgm per cent, 88 per cent, with levels of 200 mgm per cent or above. Concentrations above 200 mgm per cent were usually associated with diffuse slow activity, a typical finding being irregular high voltage 2-5 per second waves. Patients in this range were confused and inarticulate. At 180 mgm per cent, fast activity was seen to be mixed with the slow component, while at 160 mgm per cent the frequency was predominantly in the 12-25 per second range, similar to that reported by others for a moderate degree of barbiturate intoxication. The appearance of fast activity during the decline of the serum bromide level was associated with a progressive improvement in mental clarity. Below 100 mgm per cent, only occasional abnormalities were seen. Considerable individual variation was noted in the relationship between serum bromide level, degree of EEG abnormality and psychic impairment in this group of patients. However, the general correspondence between bromide concentration and incidence of EEG abnormalities confirms the previous studies of Rubin and Cohen (288), and the extreme changes observed in chronic intoxication are similar to those reported by Lennox *et al* (224) for intravenous administration of sodium bromide

The *barbiturates* have already been discussed in detail, but it might be in order

to reiterate that fast activity and to a lesser extent slow or mixed patterns are frequently seen with phenobarbital and other barbiturate therapy used in convulsive disorders M Lennox (216) has pointed out the importance of recognizing these effects in the evaluation of EEG recordings in epileptic patients, since the dysrhythmia may otherwise be mistakenly considered a sign of the disorder rather than of the treatment

The literature seems to be remarkably free of descriptions of EEG changes produced by *diphenylhydantoin* except upon preexisting dysrhythmias, which probably attests to the well-known lack of sedative effect and fair margin of safety of this substance However, the same cannot be said of the related hydantoin, *mesantoin* (3-methyl-5,5-phenyl ethyl hydantoin), which exhibits sedative side-effects in sufficient dosage Little (234) has observed consistent changes in the EEG of patients undergoing treatment with mesantoin, in contrast to the lack of EEG effect of therapeutic doses of diphenylhydantoin The changes were observed particularly with mesantoin doses of 0.4 gm /day or greater, and in order of decreasing incidence included increase in fast activity, decrease in alpha rhythm, and decrease in slow activity Only 12 per cent of their patients failed to show a demonstrable EEG effect of mesantoin The changes were closely correlated with dosage and duration of administration of the drug, but not with other factors in the patient's history

Although the oxazolidine-2,4-diones, *trimethadione* and *paraldehyde* (3,5-dimethyl-5-ethyl oxazolidine-2,4-dione), have not been reported clinically to produce consistent and significant EEG changes other than upon preexisting abnormalities, animal experiments indicate that doses of trimethadione sufficient to produce sedation cause typical sleep records, with bursts of spindles against a background of slow activity (155) In this respect trimethadione resembles the barbiturates The authors (78) and Perlstein (267) have noted a tendency in patients toward fast activity similar to that seen with barbiturates

In spite of its chemical relationship to the hydantoins, *phenurone* (phenacetylurea) produces sedative effects similar to those of the barbiturates (134) It will be interesting to note whether further EEG experience places this new anticonvulsant among those producing initial fast activity

Whether to classify *glutamic acid* as an anticonvulsant is a controversial point, even though Price *et al* (277) have reported some clinical benefit in petit mal Wager (324) failed to find that the frequency of clinical seizures in a group of 6 adult epileptics was materially influenced by nine grams of glutamic acid per day for 30 days, but reported that "electroencephalographically it was not unusual to observe an increase in recorded definition of photic drive responses and an equally noticeable removal or reduction in the recorded post-ictal psychomotor period" The nature of these changes is not immediately evident Even the largest quantities of glutamic acid which could be safely administered to laboratory animals have been found ineffective in changing either normal or metrazol-modified EEG, neither did they produce any demonstrable anticonvulsant effect (157)

From this incomplete survey of the clinically effective anticonvulsant drugs



the chief conclusion to be drawn is that abnormalities may be produced by those agents having central side-actions such as sedation, and that these changes should be considered in the evaluation of records of patients receiving anticonvulsant therapy

The second category of action of anticonvulsants, namely, their ability to improve the abnormal EEG, seems to vary with regard both to the character of the convulsive disorder and to the type of treatment. Although the EEG often shows dramatic improvement associated with clinical remission, it is also frequently found that the clinical picture improves while the EEG remains relatively abnormal (194, 220, 242, 283). These discrepancies have usually been reported in cases of grand mal or psychomotor seizures under treatment with barbiturates or hydantoins. If any general rule can be deduced from the plethora of clinical observations, it would be that frank seizures may be aborted by therapy in spite of persisting excessive discharge in the interseizure EEG. This would seem to indicate that some anticonvulsants may act to a considerable extent upon normal brain cells to protect them against invasion by seizure activity from hyperactive foci. Such a phenomenon has been noted by the reviewers (321), who observed a number of cases in which a previously diffusely abnormal EEG improved considerably under anticonvulsant therapy, but left a focus of paroxysmal discharge more sharply defined than before. Diphenylhydantoin in particular produced this effect. The most likely explanation would be that in some cases the anticonvulsant so alters the properties of normal neurones that they can no longer be secondarily involved in discharges from the epileptogenic lesion, even though the injured cells of the lesion itself continue to fire excessively.

There are also occasional reports of a salutary effect of anticonvulsant therapy upon dysrhythmias other than those associated with frank convulsive disorders. Thus, diphenylhydantoin was found by Lindsley and Henry (231) to reduce frontal slow activity without producing other EEG changes in children with behavior disorders. There was some improvement in personality but it was not well correlated with the EEG changes.

In contrast to the variable actions of hydantoins and barbiturates upon the abnormal EEG, the oxazolidine-2,4-diones have been generally reported to produce a specific depression of the spike and some dysrhythmia characteristic of the petit mal triad (pyknoplepsy, akinetic seizures, myoclonus), and there is a good correlation between EEG improvement and clinical remission. This is well illustrated by the observations of Lennox (222). He studied 100 cases of epilepsy, 59 of which had petit mal and were treated with trimethadione or paradione. Of 22 patients in the petit mal group who were rendered seizure-free, the EEGs of 72 per cent contained no spike and wave discharges, whereas none of 13 cases without clinical improvement had an improved EEG. Forty-nine patients with grand mal or psychomotor epilepsy were treated with phenobarbital, diphenylhydantoin or mesantoin. Of 16 who were rendered seizure-free, the EEGs became normal in only 31 per cent and were unimproved in 21 per cent. Of 18 patients who reported fewer attacks, 11 per cent had normal tracings. Seventy-three per

cent of those with petit mal showed agreement between clinical and EEG results, compared with 57 per cent for other types of seizures. In two thirds of the cases, when seizures were absent, improved or unimproved, the EEGs were normal, improved or unimproved, respectively. Although more specific effects of individual drugs were not noted in this study, the tabulation indicates a general tendency for seizures and EEG to follow a parallel course.

The clinical and electroencephalographic specificity of action of trimethadione in petit mal has been confirmed by a number of investigators (79, 155, 221, 265, 266, 268, 299). To a lesser extent trimethadione has been found to suppress other types of dysrhythmia. Perlstein (266) has presented evidence to show that elimination of seizure discharges not only of petit mal but also of psychomotor types and of focal fast activity may result from trimethadione therapy. Belnap *et al* (16) treated seven consecutive patients who had frequent grand mal seizures and spontaneous slow-wave dysrhythmia with a combination of trimethadione and diphenylhydantoin. The initial dysrhythmia was focal in two cases, bilaterally symmetrical frontal in three and diffuse in two. Prompt and complete clinical remission was obtained in six patients (with 1 to 9 months follow-up). Of the six patients, the EEG returned to normal in four and was improved in two.

That trimethadione may exacerbate EEG dysrhythmias in certain cases has also been noted (155), the effect is particularly striking in some patients during the first day of therapy of petit mal, and is associated with an increased seizure frequency. However, trimethadione has not been found to produce activation of latent dysrhythmias, according to Kaufmann *et al* (204) who administered 50 to 500 mgm of trimethadione intravenously in 18 patients with post-traumatic epilepsy in an attempt to localize a focus of abnormal activity. They found no noticeable effect on the EEG in any instance.

In addition to the ability of anticonvulsants to abolish convulsive discharges completely or to restrict their spatial spread over the hemispheres, certain other manifestations of protective action may be seen. For example, Lennox *et al* (224) noted, in occasional petit mal cases which responded to sodium bromide or phenobarbital, that the spike and wave episodes were sometimes reduced in frequency of occurrence, shortened in duration and distorted in pattern. With trimethadione, the reviewers have observed two interesting types of intermediate modification of the spike and wave EEG. In some patients the recurrent 3 per second pattern may be replaced by isolated single wave and spike complexes. In others, the rhythmicity may be retained but the spike component disappears, leaving episodes of regular slow waves. Finally, all investigators seem to be in agreement that in the usual case of petit mal the paroxysmal dysrhythmia is much harder to elicit by hyperventilation when the patient is under trimethadione therapy (221).

The interpretation of the favorable action of anticonvulsants upon the abnormal EEG would require a better understanding of the nature of the convulsive discharges themselves. The records of Adrian and Moruzzi (3) from single fibers of the pyramidal tract of the cat strongly suggest that convulsive discharges from the motor cortex are characterized by trains of impulses at very

high frequency, and that these may be elicited by excessive or repeated stimulation or by the application of convulsant drugs. The reviewers (316) have studied a similar phenomenon in peripheral nerve and found that pre-treatment with relatively low concentrations of the common anticonvulsant drugs protect against this effect. Part of this action was found to involve the prevention of abnormal lowering of threshold by excessive stimulation or treatment with excitant agents. In the case of diphenylhydantoin, the action was exhibited at concentrations far lower than those necessary to modify other measurable properties of nerve. If peripheral nerve data are transferrable to cerebral cortex, it is easy to see that action upon abnormal EEG discharges may occur without necessary modification of the background interseizure record or of normal function may be achieved. The question has been considered in a previous review (321).

In summary, there are two general types of action of anticonvulsants upon the EEG. On the one hand, there are effects upon the normal EEG which may be characteristic of the side-actions of the drug and are more specific for the drug than for the anticonvulsant action. On the other hand, there are the effects upon the abnormal EEG associated with convulsive disorders, these are manifested either as complete suppression of the paroxysmal dysrhythmia, or its modification by shortening the paroxysm, slowing of the frequency and disintegration of the rhythm of discharge, change in the abnormal wave form, or decrease in the evocability of the EEG signs by such activation procedures as hyperventilation. Although there are probably several mechanisms by which these ends may be achieved—mechanisms which determine the clinical specificity of the anticonvulsants—the general *modus operandi* of these drugs may be the stabilization of neuronal threshold against excessive stimulation or exciting agents in such a way as to prevent excessive facilitation and discharge of impulses at high frequency.

f Miscellaneous depressants. Other than the specific drug groups already considered, there are a number of agents, such as paraldehyde and chloral hydrate, which could also be loosely classified as central depressants. To the extent that they have sedative action, they appear to produce EEG effects like those of normal sleep (21, 136, 224), and in this respect resemble the barbiturates.

One group of central depressants which has attracted recent interest includes those which have a selective action against certain nonconvulsive motor manifestations of hyperactivity such as chorea, athetosis, tremor, etc. Among the more effective of these so-called "anti-Parkinsonism" agents is *myanesin*, which has been reported particularly effective in relieving involuntary movements of the type seen in paralysis agitans (18, 19, 295). Stephen and Chandy (305) recorded the EEG tracings during the intravenous administration of 30 mgm/kg, a dose which abolished involuntary movements in paralysis agitans and stopped intractable pain of thalamic origin for short periods of time. There was no significant alteration in EEG activity except an increased alpha rhythm in certain cases with increased nervous tension, an observation suggesting general relaxation. No slow waves indicative of possible cortical depression appeared, but in one case "abnormal waves" were recorded from the base of the brain, they disap-

peared with remission of clinical signs and symptoms Gammon and Churchill (118) found no alteration of normal EEG patterns with doses of myanesin which abolished the tremors of Parkinsonism or suppressed choreoathetotic movements. However, in six cases of petit mal without generalized seizures, the spike and wave discharges were abolished. Convulsive states with focal cortical abnormalities and two cases of "petit mal variant" associated with generalized seizures, supposedly the result of brain damage, were unaffected by myanesin.

Experimental animal studies (317) indicate that quantities of myanesin sufficient to produce generalized muscle relaxation, particularly of the hindlimb and abdominal musculature, may cause the appearance of sleep activity in the EEG, but the extent of loss of muscular tone is considerably greater than that seen with an amount of a rapidly acting barbiturate causing an equivalent EEG change. That the EEG changes may be irrelevant to the basic action of centrally effective muscular relaxants is illustrated by benzimidazole, which can produce an extreme loss of tone with no observable change in the EEG (147). Observations on peripheral nerve and on brains of animals indicate that these agents act by raising threshold rather than by altering the membrane potential, but the precise mechanism of their apparent specificity as muscle relaxants is still to be elucidated (317).

*2 Predominantly excitant drugs* The drugs which exhibit a predominantly excitatory effect upon the central nervous system vary widely in the degree and kind of excitation they produce. Some agents, such as metrazol and picrotoxin, may produce full convulsive seizures and corresponding EEG signs of paroxysmal hyperdischarge, whereas others, such as amphetamine, produce a relatively mild analeptic effect which is best observed against a background of central depression produced by other drugs. Thus it is not surprising that there is no uniform EEG manifestation of the action of central stimulants.

*a Strychnine* Strychnine is among the most ancient of the central stimulants and has received experimental attention and wide clinical usage far out of proportion to its therapeutic value. Its most striking central action in man and animals is the production of hyperexcitability of the spinal cord so that stimulation which would ordinarily produce only localized responses, or even inhibition of reflexes, is capable of evoking massive and diffuse tonic spasms of the body musculature (148). However, its excitant effects are not restricted to the cord but are also seen at higher levels.

The EEG manifestations of strychnine poisoning have not been adequately investigated in man (136), but considerable information has been derived from work with experimental animals. Bremer (38) reported that topical application of strychnine to the cortex of the cat at first produced an amplification of the preexisting spontaneous rhythm, but eventually this background activity became weaker and high voltage, highly synchronized spikes appeared, initially in isolation and then in trains. As with other types of convulsive discharge, these were followed by a period of functional depression lasting many minutes. Adrian and Moruzzi (3) found that the strychnine spikes recorded from the motor cortex of the cat after topical application of the drug were associated with bursts of

impulses in individual pyramidal fibers at frequencies greater than 1000 per second. After systemic administration of strychnine to cats and rabbits, Heinbecker and Bartley (177) recorded an initial increase in frequency and amplitude of cortical activity, followed by the appearance of high voltage synchronized discharges. The threshold for responses evoked from the cortex by sciatic stimulation was somewhat reduced by strychnine, and the late components of the evoked potential were dramatically increased in size. In addition, the period of facilitation following a single response was considerably prolonged. These cortical effects could be abolished by calcium gluconate.

The early literature on the mechanism of action of strychnine has been reviewed by Dusser de Barenne (90a), whose own work seems to demonstrate a selective excitant action of strychnine upon cell bodies. This action has been widely exploited by Dusser de Barenne and his colleagues (cf. 246) in neuroanographic mapping of the interrelation between various cortical areas and subcortical structures, since after topical application of the drug the excessive firing manifested by strychninized cell somata is propagated to other parts of the brain by the terminations of the activated neurones.

Systematic investigation of the actions of strychnine on various levels of the nervous system has been made by Heinbecker and Bartley (177). They noted two significant effects of relatively low concentrations upon peripheral nerve, namely, a lowering of threshold and a decrease in the degree of accommodation. In isolated autonomic ganglia they observed an increase in excitability and a great prolongation of the period of enhanced excitability following an inadequate preganglionic volley. Reflex discharges obtained from the spinal cord were characterized by a great prolongation of the period of firing following a single afferent shock. The effect was greatest when both dorsal and ventral quadrants were strychninized, it was still apparent when the ventral surface only was treated, but was absent when the drug was applied only to the dorsal horn. These results on the cord were essentially similar to the findings of Dusser de Barenne (90a), who had also shown a hypersensitivity of the body segments innervated by the dorsal surface of a strychninized portion of cord, but an absence of tetanic reflex contractions unless the ventral surface was also treated. Heinbecker and Bartley demonstrated an increase in the frequency, amplitude and duration of discharges from the pacemaker neurones of the limulus heart treated with strychnine.

Since strychnine acts upon many types of neurones both to cause a moderate increase in excitability and to prolong the duration and increase the frequency of trains of impulses from the excited cells, possibly by reducing accommodation, it does not seem necessary to postulate additional mechanisms for the appearance of strychnine spikes in the EEG. Each strychnine spike may be considered to be a synchronized composite of high frequency discharges from individual neurones with periods of recovery intervening between the summated bursts. It would be interesting to know to what extent the high degree of synchronization shown in each burst is predicated on a loss of cortical inhibition, particularly since the failure of inhibition is known to be so pronounced at the spinal level.

Also, the role of loss of accommodation in this phenomenon would be worth further study

b Metrazol Metrazol differs from strychnine in having its most pronounced effects upon the higher centers of the brain rather than upon the cord, and in producing complete tonic-clonic convulsions rather than brief tonic spasms Its chief valid uses in the past have been in the shock therapy of psychoses and as a cerebral and respiratory stimulant to combat the effects of central depressant drugs Of greater interest to electroencephalographers is the recent use of metrazol as an "activating" agent for latent dysrhythmias Kaufman *et al* (204, 205) have reviewed various methods now used clinically to elicit abnormalities in the EEG, particularly in patients with suspected convulsive disorders Metrazol has been used more than other convulsant drugs for the purpose of provoking paroxysmal discharges in susceptible individuals, and at least some epileptic patients have abnormally low thresholds for metrazol activation Ziskind and Bercel (348, 349) have been foremost in using the minimal threshold quantity of metrazol as a diagnostic procedure to elicit EEG abnormalities The typical minimal activation pattern was found to consist of high voltage 4-5 per second oscillations occurring in bursts Unfortunately these bursts tend to be generalized even in the presence of well-defined foci, and the technic is of no unusual advantage in localization

Because of the current interest in activation procedures, it is worthwhile to mention some of the more detailed observations made by the above investigators A study of 25 epileptics with a preliminary normal EEG (348) showed that the average minimal dose of metrazol for evoking EEG changes was 2.3 cc of a 10 per cent solution, for non-epileptics, 3.4 cc However, there was considerable overlapping of the two groups Records obtained from epileptics before and after the administration of 1 or 2 doses of 0.1 gram of phenobarbital and 0.1 gram of diphenylhydantoin showed a definite rise in threshold In patients with spontaneous larval "spike and wave" attacks, the paroxysmal record was very sensitive to the intravenous injection of either sodium phenobarbital or trimethadione, which obliterated or greatly diminished the abnormal discharges Hyperventilation and sub-convulsive doses of metrazol readily reproduced these larval attacks and restored the same pattern which was present in the preliminary record Kaufman *et al* (204, 205), in a study of metrazol activation in post-traumatic epilepsy, administered 2 cc of a 10 per cent solution intravenously as rapidly as possible during EEG recording, and produced localized alterations in the cortical rhythms in 60 per cent of 97 patients Focal changes included slow waves and single or multiple spikes, giving the appearance of a localized EEG seizure Ten per cent of patients showed generalized alterations consisting of single or multiple slow waves or spikes appearing simultaneously from several areas of the head A small percentage of the patients examined experienced sensory aura or motor prodromata The seizure induced by metrazol had the characteristics of the patient's ordinary attacks In approximately one-half the attack remained confined to one portion of the body as a focal convulsion Patients on anticonvulsant therapy showed a lower incidence of activation, and none of them had

clinical convulsions with the dosage used Intramuscular administration was found to be less effective than intravenous administration in producing clinical seizures and in localizing an epileptogenic focus

The use of metrazol in convulsive therapy has also led to sporadic studies of the associated EEG changes (84, 306) These are usually reported to be similar to the patterns occurring during spontaneous convulsions Post-seizure EEGs taken for several months following a series of metrazol convulsions have shown residual disturbances in as many as 50 per cent of patients, associated with evidences of impaired cerebral function lasting sometimes as long as six months (207, 227) The reported EEG abnormalities include slow activity ranging down to 3 per second, bicuspid and dicrotic waves, spike and wave formations and greatly increased amplitude Presumably these persistent changes are the result of the cumulative effects of a series of seizures however produced, and have no special significance for the action of metrazol

The EEG changes produced by metrazol in experimental animals have received considerable attention and have found a practical application in investigations of the protective action of anticonvulsants (155, 315, 317, 320, 322, 348, 349) In rabbits, approximately one half of a convulsive dose of metrazol produces a dysrhythmia characterized by bursts of smooth 5 per second waves of high voltage, usually with a small spike component which gives the record the superficial appearance of a petit mal spike and wave burst The spike is more prominent in rats and monkeys and is the dominant feature of the discharge in cats In rabbits the metrazol threshold for this type of discharge is lowest for the auditory cortex, next for the visual cortex, and higher still for the rostral sensorimotor cortex When the bursts occur intermittently, they are usually associated with quiescence of the animal, and are easily abolished during spontaneous movements or sensory stimulation Larger doses of metrazol lead to the development of a continuous dysrhythmia of this type, but until the discharge has become continuous in the rostral cortex there are no overt convulsive manifestations and no gross evidences of excitation except for hyperpnea The onset of a seizure is usually heralded by the appearance of groups of high voltage spikes particularly in the motor cortex, associated with clonic movements, and this is then followed typically by the development of a full clinical and EEG tonic-clonic seizure

Such an EEG pattern provides an interesting preparation for the study of anticonvulsant drug action (320) Trimethadione has been found highly effective and specific in raising the metrazol threshold for these discharges, but all the barbiturates which have been studied are also active Benzimidazole, in spite of its ability to raise electrical threshold in the brain, is relatively ineffective against the metrazol discharges The reviewers have found diphenylhydantoin, glutamic acid, and respiratory and metabolic acidosis all relatively ineffective against metrazol Amphetamine increases the sensitivity of the brain to metrazol, although it is not in itself capable of producing this type of dysrhythmia

The reviewers have studied cortical responses to electrical stimulation in an analysis of the action of metrazol (315, 320, 322) The characteristic EEG discharge may be evoked by electrical stimulation of the brain even when the dos-

age of metrazol is too small to alter the spontaneous record. During continuous intravenous injection of metrazol the single slow wave component of the normal evoked discharge can be seen to grow progressively in amplitude and then to become repetitive. These changes are associated with a progressive lowering of threshold for the evocation of EEG discharges, nonconvulsive movements and seizures. At a point just short of the occurrence of seizures, the electroshock threshold may be reduced to about half, and single shocks, which ordinarily do not produce seizures no matter how high the voltage, now may precipitate a convulsion. The development of the preconvulsive state is also associated with evidences of loss of local sign for stimulation of motor points and wide irradiation of sensory discharges. For example, auditory click stimulation which ordinarily gives small local responses confined to the primary auditory areas, now produces diffuse subconvulsive EEG bursts and may even precipitate seizures.

Thus the characteristic features of metrazol action as deduced from EEG observations include the development of repetitiveness, increased amplitude of discharges, a moderate lowering of threshold, and widespread irradiation. In contrast there seems to be little effect on the time relations of the recovery cycle of the cortex.

These same features are also seen to some extent when metrazol is used in animals already depressed by trimethadione or barbiturates. For example, metrazol increases the voltage, frequency, duration and rate of recovery of cortical responses evoked by sciatic or cortical stimulation in cats and rabbits under deep barbiturate anesthesia. In light anesthesia metrazol increases the frequency and also the voltage of the "spindle" bursts, amplifying particularly the surface-negative component of these discharges, an indication of increased synaptic transfer within the cortical layers. The antagonism of metrazol is more specific for trimethadione than for the barbiturates, so that the various EEG stages of anesthesia and sleep produced by trimethadione can be progressively overcome with increasing doses of metrazol.

The authors (317) have been unable to find any action of metrazol on peripheral nerve corresponding to its excitant effect upon the cerebrospinal axis. With unphysiologically high concentrations (30 mMol/l or more) an increase in threshold and other depressant effects may be observed. In this respect metrazol differs from strychnine, and the difference may have relevance to the more selective action of metrazol on higher centers. If further definition of the mechanism of action of metrazol is to be found, it might therefore be sought most profitably at the level of the cerebral cortex itself.

c **Picrotoxin** Picrotoxin is a convulsant which does not differ significantly from metrazol in its effects upon the nervous system except insofar as its onset of action and rate of destruction are somewhat slower. Its clinical use has been largely restricted to the treatment of barbiturate poisoning (148), and its EEG effects have not attracted the same wide interest as those of metrazol. Sporadic observations on laboratory animals (311, 317) indicate that it produces the same EEG changes as does metrazol.

d **Camphor** Camphor, which resembles metrazol and picrotoxin in produc-



ing seizures by a higher central action, is not clinically used for its convulsant or analeptic properties, and its EEG effects have therefore been of little interest. Lennox, Gibbs and Gibbs (224) observed that after the intravenous injection of small amounts of camphor, the brain potentials increased in amplitude, becoming progressively larger and more frequent until a convulsion occurred. In one patient in their series, a short but typical petit mal seizure developed. In another patient with a history of seizures, psychomotor attacks as well as generalized seizures were produced. These observations might suggest camphor for use in EEG activation procedures, but it is unlikely that this drug possesses any advantages over metrazol.

e. Amphetamine. Among the central stimulants, amphetamine has received increasing attention because of its popular use in allaying drowsiness and in dulling the appetite of the obese. Its reported effects on the EEG of normal patients have included an increase in percentage time alpha, according to Rubin *et al.* (293), and a shift toward higher frequencies in the EEG spectrum as studied by Gibbs and Maltby (141). Although Cutts and Jasper (70) failed to find any definite effect of amphetamine in the EEG of behavior problem children who showed clinical improvement during treatment, Lindsley and Henry (231) in a similar group reported some reduction in slow frontal abnormalities, and an increase in frequency and decrease in amplitude of the normal alpha rhythm. A more specific effect of amphetamine on EEG abnormalities has been observed in petit mal cases by Golla *et al.* (146) and Livingston *et al.* (235), who found that the characteristic 3 per second spike and wave pattern was often abolished by treatment. The latter investigators did not find a close correlation between EEG improvement and clinical remission in their series, and noted that amphetamine was less effective when the EEG dysrhythmia deviated from the classical petit mal type. These effects of amphetamine should probably not be attributed to a specific action of the drug in changing cortical parameters, since petit mal discharges are notoriously easily suppressed by procedures which increase alertness and activity.

The reviewers (317) have noted no outstanding changes in the EEG or thresholds of experimental animals treated with amphetamine. Interestingly enough, the petit mal-like dysrhythmia produced by subconvulsive doses of metrazol was found to be exacerbated rather than improved by amphetamine. M. Lennox and Ruch (217a) have observed that post-seizure abnormalities of the 2-3 per second type which usually appear in monkeys during recovery from electroshock convulsions may be abolished by pretreatment with amphetamine. The drug also produced a more rapid recovery from post-seizure depression.

The mechanism of action of amphetamine is far from understood, it is probably unrelated to its sympathomimetic action (148). The work of Maling and Acheson (241a) would indicate, at least to the reviewers, that amphetamine can compensate to a considerable degree for the effects of various central nervous lesions, possibly by replacing through increased internuncial function the loss of facilitation from the centers destroyed. That the mechanism is considerably different from that of the convulsant drugs may be inferred from the failure of

amphetamine to produce seizures or subconvulsive dysrhythmias in laboratory animals, and its ability to combat drowsiness without interfering with the anti-epileptic action of such agents as phenobarbital in the treatment of convulsive disorders

f Xanthines The xanthines, although widely used as central stimulants, have not been favored with extensive observations of their EEG effects Gibbs and Maltby (141) reported that caffeine causes a shift in the human EEG frequency spectrum toward faster activity This shift included both an increase in frequency of the alpha rhythm and increased voltage in the 14-30 per second range Swank and Foley (311) noted that caffeine and aminophylline differed from metrazol and picrotoxin in having no effect upon the EEG in doses which were definitely analeptic against the respiratory depression induced by barbiturates

g Cocaine and procaine Although useful primarily as local anesthetics, procaine and cocaine have important central nervous actions which are attracting increasing attention—procaine because of its use as an analgesic by intravenous administration, and cocaine because of the public health problem created by its chronic use particularly among the hunger-ridden Indian miners of the Andes Both may produce extreme excitation and convulsions on systemic administration The EEG correlates of their central action have not been extensively studied Berger (21) observed an increase in the amplitude of the alpha waves without change in frequency following the administration of cocaine to 10 subjects Rubin *et al* (293) administered 50 mgm of cocaine subcutaneously to each of 11 subjects and found a decrease in the percentage of alpha activity, without significant change in the alpha frequency or in the amount of slow activity The reviewers (317) have recorded the effects of intravenous procaine on the EEG of the rabbit and observed changes essentially similar to those obtained with metrazol, including the early appearance of high voltage 5 per second spike and wave bursts Procaine differs from metrazol in increasing rather than decreasing the threshold for evoked cortical responses, but both cause repetition and increased voltage of the evoked responses as well as widespread irradiation As to the basic mechanism of action of the local anesthetics, it is well known from the classical investigations of Gasser and Erlanger (118a) that conduction is first blocked in the small and unmyelinated fiber groups, and it might be expected that the small neurones of the brain would be the earliest to fail after systemic administration How this mechanism could operate to permit irradiation and seizures remains to be determined The important factor in conduction block by the local anesthetics has been shown to be increase in threshold rather than depolarization (17, 25, 238, 323) This raises the possibility that a threshold-increasing drug may cause seizures by selective depression of inhibitory systems Further investigations on the apparent paradoxical action of procaine might do much to clarify the mechanism of origin of subconvulsive EEG dysrhythmias produced by this and other convulsant drugs

h Mescaline Mescaline, a pharmacologically active substance derived from cactus, is capable of producing bizarre psychic effects and hallucinations (148),

it is also occasionally used in the laboratory as a convulsant (123) Rubin *et al* (293) have examined its EEG effects in man. The oral administration of 300 mgm of mescaline in divided doses to a group of seven schizophrenics produced extreme states of anxiety, correlated with a 25 to 30 per cent increase in the frequency of the alpha rhythm. Chweitzer (59) reported that, in a normal individual during mescaline intoxication, there was a reduction in the amplitude of the alpha waves, with prolonged intervals of inactivity associated with the hallucinations. These observations are of little help in interpreting the peculiar psychic effects of mescaline, and the literature has been more concerned with the exotic features of mescaline intoxication than with its mechanism of action.

1. DDT. Occasional cases of poisoning in animals and man by the insecticide DDT (Dichloro-diphenyl-trichloro-ethane), and the unusual character of the seizures induced in animals, prompted Crescitelli and Gilman (66) to study the alteration in the electrical activity of the cerebellum and cerebral cortex resulting from the intravenous administration of DDT emulsions in cats and monkeys. In animals which had been treated previously with small doses of sodium pentobarbital to suppress the convulsive activity of DDT, the cerebellar rhythm progressively increased in magnitude over the course of one to two hours, to a level five times that recorded during the control period. The pattern remained essentially unchanged, except for a slight increase in frequency. Cortical activity increased somewhat in magnitude and frequency, but the chief effect, especially in cats, was the conversion of previously irregular 8-12 per second pattern into an almost continuous and regular rhythm at the same frequency or slightly higher. With further action of DDT, episodic convulsive activity was recorded from both the cerebellar and cerebral cortex, preceded by fast waves. These fast discharges were completely synchronized in the motor cortex and cerebellum, in monkeys they were surface positive at the motor cortex and either surface negative or diphasic, with an initial negative deflection, at the cerebellum. The fast activity increased in magnitude and frequency until eventually periodic electrical seizures appeared in both the cerebellum and cerebral cortex, similar to the electrical pattern observed in major seizures in man or that following the administration of convulsive drugs or electrical stimulation of the cortex in animals.

A detailed analysis of the preconvulsive fast waves in monkeys revealed that they were most prominent in leads from areas 4 and 6. With leads placed progressively away from the motor cortex, the fast waves became less prominent. From the cerebellum, these discharges were recorded most prominently from the pyramic vermis and portions of the lobulus simplex. In most instances, the lobulus ansiformis was silent or relatively inconspicuous. The ansiform lobe and the vermis of the cerebellum, like the cerebrum, participated in the periodic "tonic-clonic" electrical manifestations of DDT.

The authors concluded that on the basis of polarity, the cerebellar-cortical synchronization could only be explained as the result of efferent impulses from cerebellum to cortex. However, they were unable to explain the EEG findings on the basis of known connections between the cortex and various parts of the cerebellum, and have suggested the possibility that both the cerebellum and motor

cortex were being simultaneously activated by impulses from a mass of neurones linked to both areas. It would be interesting indeed if agents could be found which have a selective convulsant action on particular subcortical centers, but the physiological mechanism would still require as careful analysis as that of the more diffusely acting convulsants.

*B Substances used primarily for other than central nervous system effects*

1 *Autonomic agents* During the past decade, considerable attention has been given to the role of acetylcholine in central synaptic transmission, and the search for a universal chemical mediator has generated many investigations of the effects of cholinergic and other autonomic drugs on the EEG. Indeed, the ability of any cholinergic drug or blocking agent to change the EEG is all too frequently seized upon as verification of an essential central role of acetylcholine, even when the most unphysiologically high concentrations are used. Despite these excesses, there is no doubt that acetylcholine and many agents which modify its actions or alter its rate of destruction may have profound effects on central nervous function, with corresponding repercussions in the EEG. The problem of the significance of acetylcholine in cerebral function has been recently reviewed by Feldberg (106) and Whitteridge (337), among others.

a *Acetylcholine* There has been no dearth of empirical observations on the effects of acetylcholine on the EEG, particularly of animals. Sjöstrand (300) found that acetylcholine topically applied to the cortex increased the amplitude of strychnine and eserine spikes and caused increased frequency and grouping of waves. Stronger concentrations or repeated applications inhibited the spikes. Bonnet and Bremer (28) and Bremer (41) injected small doses into the carotid artery of midbrain-transected cats and found increased amplitude and frequency of the dominant waking rhythm, and increased after-discharge following auditory stimuli. Larger doses had a depressant effect. Moruzzi (257) confirmed these observations in the rabbit, and also noted a decrease in electrical threshold. Miller *et al* (254) found a desynchronizing effect of topically applied acetylcholine on the EEG of the cat, and a strychnine-like effect on the previously eserinizied cortex. Chatfield and Dempsey (53) saw no effect with acetylcholine alone, but after prostigmine, which itself caused bursts, acetylcholine produced increased spiking and low voltage fast activity. The latter remained even after isolation of the cortex from the thalamus. Williams (344) found that acetylcholine and carbaminoylecholine produced petit mal-like activity in susceptible patients. Intracisternal injection of acetylcholine in cats and man produces generalized convulsions (45, 107, 114), as does intravenous injection of large doses (61, 174, 287). That these seizures are not necessarily due to direct action on central nervous system is suggested by the recent report of Ajmone-Marson and Fuortes (5) who studied the convulsions elicited in dogs by intravenous acetylcholine. The seizures were associated with flattening of the EEG rather than with convulsive discharges, and the changes were similar to those of asphyxiation, such as might be expected from the cardiac arrest produced by acetylcholine intravenously. The investigators give the same explanation for the hyperactivity of the spinal cord

seen under these conditions, and conclude that the observed convulsive movements are clearly not of cortical origin. However, this objection need not apply to focal or intracisternal injection of acetylcholine and other choline esters, and the work of Brenner and Merritt (45) and Forster (114) suggests that EEG and motor manifestations of these drugs may be elicited directly from the cortex.

In any consideration of the suspected functional role of a chemical substance, the relationship between the amount normally present and the amount required for the functional change should be in reasonable agreement. Such a comparison has been made by Bornstein (30), who found that acetylcholine perfused over the cortex of dogs and cats produced high amplitude sharp waves in low concentrations (1  $\mu$ gm per cent), whereas flattening of the EEG occurred with higher concentrations (2  $\mu$ gm per cent or more). Both of these EEG effects were also seen after experimental trauma to the head, the trauma was shown to result in the appearance of "free" acetylcholine in the cerebrospinal fluid in concentrations up to 9  $\mu$ gm per cent. The EEG and behavioral effects of both trauma and intracisternal acetylcholine could be abolished by the subcutaneous administration of atropine sulfate, 0.5 to 1.0 mgm/kg. Bornstein suggests that "free acetylcholine" may be one of the physiological factors underlying the acute paralytic and excitatory phenomena of cerebral concussion and more severe craniocerebral injuries.

b. *Pilocarpine*. In contrast to acetylcholine, pilocarpine in systemically effective concentrations was without EEG action in the patients examined by Williams (344) and in the cats studied by Miller *et al* (254).

c. *Anticholinesterases*. If acetylcholine is present in the central nervous system in sufficient quantities to exert some physiological action, regardless of the essential role of this action, then those agents which retard the hydrolysis of acetylcholine by cholinesterase might be expected to produce functional central changes and associated EEG signs similar to those elicited by exogenous acetylcholine. In general, this appears to be the case, and the few reported differences in the action of the various anticholinesterases may tentatively be ascribed either to side-effects unrelated to cholinesterase inhibition or to differences in enzyme specificity or ability to penetrate cells. The most complete observations to date have been made with the reversible anticholinesterases eserine (physostigmine) and neostigmine and the irreversible inhibitor di-isopropyl fluorophosphate (DFP).

(1) *Di-isopropyl fluorophosphate*. Extensive studies on the effects of di-isopropyl fluorophosphate (DFP) in man have been carried out by Grob and his associates (169, 170, 171, 175). The daily intramuscular injection of 1 to 2 mgm of DFP in 23 subjects (19 normals, 4 with myasthenia gravis) resulted in increased electrical activity in 17, manifested by greater variation in amplitude, increased frequency, more beta activity, more irregularities in rhythm, and the intermittent appearance of abnormal high voltage 3-6 per second waves similar to those frequently seen in patient with convulsive disorders. These alterations were usually most marked in the frontal regions and were increased by hyperventilation. The most striking changes were observed in those subjects who showed greatest lability of pattern in their control records. The EEG signs ap-

peared after 2 to 7 days of DFP administration, usually following the onset of central nervous symptoms. After cessation of DFP administration, symptoms disappeared in 1 to 4 days, whereas EEG changes persisted in diminishing degree for eight to 42 days.

The EEG effects of DFP could be abolished temporarily by intravenous administration of atropine, or delayed considerably by chronic atropine therapy. In contrast, intravenous injection of neostigmine, curare or *d*-tubocurarine resulted in no change in the central nervous symptoms or EEG signs of DFP poisoning, although the doses of curare and *d*-tubocurarine were sufficient to cause weakness of the facial and ocular muscles, and the amount of neostigmine was adequate to produce gastrointestinal effects.

The onset of central nervous symptoms and the appearance of EEG changes after DFP could usually be correlated with a depression of red blood cell cholinesterase activity to 70% and 60% respectively of the original activity, when DFP was administered over a relatively short period (up to 3 days). However, when DFP was administered over a longer period of time or when administration was stopped, correlation between central nervous effects and erythrocyte cholinesterase activity no longer existed. The onset and severity of symptoms and EEG changes bore no relationship to the cholinesterase activity of the plasma.

The mode of action of DFP was considered by Grob and associates to be the irreversible inhibition of the central nervous system by DFP cholinesterase. The persistence of the EEG changes for as long as three to four weeks after the last dose of the drug probably reflects the slow regeneration of brain cholinesterase. The lack of correlation of DFP effects with erythrocyte cholinesterase activity after the first few days presumably is due to the different rates of regeneration of the cholinesterases in the central nervous system and in the red blood cells. Because neostigmine could not be given in amounts sufficient to duplicate or potentiate the central effects of DFP without causing gastrointestinal distress, the authors conclude that the greater lipid solubility of DFP favors its selective action upon the central nervous system. However, the possibility of a difference in enzymatic structure or in rate of regeneration at the two sites should not be automatically excluded.

The EEG effects of DFP have been further analyzed in experimental animals. In the cat and monkey Wescoe *et al.* (335) observed an increase in frequency and a decrease in amplitude of cortical discharges within one minute after intravenous injection of DFP. These effects could be abolished or prevented by the intravenous administration of atropine or scopolamine. Intracarotid injection of DFP in the curarized rabbit was found by Himwich and his colleagues (115, 172) to result in a slight decrease in fundamental frequency accompanied by a decrease in voltage, followed by the appearance of high amplitude discharges. These occurred first on the side of injection and then became generalized and persistent, resembling the pattern of *status grand mal*. Small doses of atropine administered after the high amplitude discharges had become established restored the pattern to normal, larger doses of atropine injected prior to the administration of DFP prevented the high amplitude waves, and a further increase in dosage eliminated

all abnormal responses. The electrical changes induced by DFP were associated with a severe depression of anticholinesterase activity of the cerebral hemispheres, the cerebellum and the brain stem, but in no instance was the cholinesterase activity completely eliminated.

(2) *Eserine* and *neostigmine* The reversible anticholinesterases also have been shown to cause EEG changes resembling those seen with *acetylcholine*. Sjostrand (300) reported a potentiation of strychnine spikes by *eserine* in the EEG of experimental animals. Miller *et al* (253, 254) observed a reduction of voltage and a dissociation of previously synchronized cortical waves in the EEG of the rabbit and cat after topical application of *eserine*, and Chatfield and Dempsey (53) observed a similar initial depression of voltage with *neostigmine*. Darrow *et al* (73) found that *eserine* prevented hyperventilation-induced slow waves in cats whose parasympathetic innervation to pial vessels had been cut.

In patients with petit mal, Williams and Russell (345) have demonstrated an interesting difference between *eserine* and *neostigmine*, the former decreasing and the latter increasing the incidence of spike and wave discharges. To explain this paradox they have invoked Schweitzer's (296) schema in which *eserine* (as a fat-soluble tertiary ammonium base) excites by penetrating the neurone soma, whereas *neostigmine* (as a water-soluble quaternary base) inhibits at the synapse. This explanation is somewhat compromised by the many and apparently inconsistent similarities and differences in the action of the two drugs as reported by various other investigators (106).

d *Atropine* Of the various drugs which can inhibit the action of *acetylcholine* upon effector cells, *atropine* is the only one which is consistently reported effective in studies on central neurones. Feldberg (106) has reviewed a large body of evidence for a blocking action of *atropine* against the effects of cholinergic and anticholinesterasic agents at all levels of the central nervous system. That the blocking action of *atropine* is often exhibited in concentrations which alone have little or no effect on normal central functions suggests no vital role of endogenous *acetylcholine* in the ordinary activities of the brain. However, reports of direct actions of *atropine* upon the EEG have not been lacking. Grob *et al* (169) found that the intravenous administration of 1.2 mgm of *atropine* to 39 normal subjects resulted in an immediate decrease in frequency and voltage, with decreased beta and increased alpha rhythm, a decrease in the irregularities of the rhythm and a decrease in the appearance of abnormal slow waves during hyperventilation in over one third of the subjects. The same dose of *atropine* in 16 subjects with a history of grand mal and with an EEG pattern "characteristic of this disease" resulted in some reduction of abnormal discharges and of amplitude in one half of the subjects. These changes, which occurred immediately following intravenous injection, were in the same direction as those occurring both in control subjects and in those who had received DFP, but were less marked than in the latter group and were dramatic in only one case.

Increased EEG activity following DFP was inhibited by *atropine* in all 17 subjects studied by Grob *et al* (169). Intravenous injection of 1.2 mgm of *atropine* resulted in an immediate decrease in potential and frequency, with decreased beta

and increased alpha rhythm, a decrease in the irregularities of the rhythm, and decrease in the incidence of abnormal waves at rest and during hyperventilation. Daily administration of atropine concomitant with daily administration of DFP delayed the onset of both central nervous symptoms and EEG changes, the former for a few days and the latter for as long as three to four weeks, at which time the erythrocyte cholinesterase activity had been reduced to 33% of the original activity. Some of the subjects who had received atropine and DFP daily for several weeks had pronounced central nervous symptoms despite the absence of EEG changes. Withdrawal of atropine was followed within several days by the appearance of EEG abnormalities.

Williams (343, 344) found that spontaneous and hyperventilation-induced spike and wave paroxysms were sometimes blocked by atropine in patients with petit mal, and that atropine prevented the exacerbation of these attacks by acetylcholine and other cholinergic agents. On the other hand Darrow *et al* (74) observed an increase in hyperventilation-induced slow waves and in various resting abnormalities in patients who were given sufficient atropine to cause cardiac acceleration, although the EEG was sometimes improved when the systemic actions of atropine were incomplete.

Not all investigators have been able to demonstrate an atropine blockade of the EEG effects of cholinergic agents. In the studies of Forster (114), atropine failed to prevent the initial depression and ultimate paroxysmal EEG effects of intracisternally or topically applied acetylcholine in the cat. Brenner and Merritt (45) also were unable to prevent cholinergically-induced convulsive EEG discharges with atropine. On the other hand, Chatfield and Dempsey (53) abolished acetylcholine-induced spikes in the EEG of the cat by giving atropine intravenously. Miller *et al* (254) also found that spikes occurring after local application of eserine and acetylcholine were suppressed by intravenous injected or locally applied atropine. Atropine itself produced unusual EEG abnormalities when applied locally. Darrow *et al* (73) observed that after a sufficient dose of atropine, hyperventilation caused the appearance of high voltage slow waves in cats. They also found that atropine restored the hyperventilation dysrhythmia when it had been blocked by eserine. Finally, Bornstein (30) observed that atropine prevented the EEG effects of both concussion and exogenous acetylcholine in cats.

c. Curariform agents. If central synaptic transmission were dependent upon the same chemical mechanism as that in autonomic ganglia and the neuromyal junction, curare would be expected to have significant central blocking actions. However, a definitive experiment demonstrating the lack of cerebral effects of curare in man was conducted by Smith and his colleagues (301). These observers administered a total of 500 units of *d*-tubocurarine intravenously to a healthy trained adult during a period of 33 minutes, an amount at least  $2\frac{1}{2}$  times that required to produce complete respiratory paralysis, and adequate for complete skeletal muscular paralysis. No impairment of consciousness, memory or sensorium was observed, likewise, no evidence was obtained of central stimulant, depressant or analgesic properties of curare. The amplitude, frequency and per-



centage time alpha of the EEG remained unchanged, and blocking of the alpha rhythm by pattern vision remained normal. A total of 3.5 mgm of neostigmine, given intravenously to facilitate recovery, also failed to alter the EEG.

Some animal observations have suggested central actions of curare. McIntyre (247, 248, 249) reported that *d*-tubocurarine modifies the electrical activity of the brain of dogs lightly anesthetized with various barbiturates. He noted an initial transient increase in activity simultaneously in the occipital, parietal and frontal regions, with as much as a three-fold increase in voltage. The frequency was irregular, sometimes exhibiting spikes followed by low voltage 100/sec. activity. Immediately thereafter, electrocortical activity was depressed, even with doses insufficient to prevent diaphragmatic respiration. With larger doses the electrical activity of the frontal areas was decreased very rapidly and the initial transient stimulation was very brief. Depression of the frontal areas occurred before peripheral paralysis. Since adequate details of experimental technique were not presented, it is not clear whether the barbiturates were in part responsible for the results, or whether some degree of hypoxia occurred.

When the complicating factor of respiratory depression and secondary anoxia is avoided by adequate artificial ventilation, most investigators find no effect of curare on the EEG (102, 103, 143, 144, 317). Everett (102) observed no demonstrable effect on the EEG of cats, rats and rabbits, given curare in doses 5 to 50 times greater than necessary to produce respiratory paralysis. Furthermore, a convulsive dose of metrazol after curarization produced a typical seizure discharge although all motor manifestations were absent and the electromyograph showed no spikes. Also, no change in electroshock threshold is produced by curare. On the other hand, Everett (103) found that curare given intracisternally could produce violent convulsions in rabbits. The effect was similar to that of penicillin, which causes convulsions following local application to the brain but not following intravenous administration. There have been no systematic studies of the possible protective action of curare against central effects of cholinergic drugs equivalent to the investigations conducted with atropine. However, the incidental use of curariform agents to immobilize animals for EEG records apparently does not militate against the characteristic effects of acetylcholine or cholinergic agents (73).

One group of investigators (105, 273) has consistently found blocking effects of curariform drugs on the EEG of the frog, in doses equal to or more than necessary to cause a reversible neuromuscular paralysis. Curare, *d*-tubocurarine chloride and dihydro-beta-erythroidine hydrochloride all caused a depression of cortical activity which could not be restored by neostigmine, strychnine or picrotoxin. Quinine ethochloride, nicotine and thiamine likewise flattened the EEG after an initial acceleration of frequency, and neostigmine was of no avail in reviving the silent hemispheres. A more analytical reinvestigation of these findings would be of interest.

**f Epinephrine** The attention given to the action of epinephrine upon the EEG has been disproportionately small in comparison with that lavished upon the more popular neurohumor acetylcholine, despite the fact that the latter substance

has not been conclusively demonstrated to produce central changes other than through a vascular action. Among sporadic studies with the adrenergic mediator are those of Grinker and Serota (168) who recorded increased fast and random slow activity with disappearance of the alpha rhythm in the hypothalamus as well as the cortex of patients receiving intravenous epinephrine. Schizophrenic patients were found less responsive than controls to the psychic and EEG effects of epinephrine. The newer sympathetic blocking agents, especially dibenamine, have been shown to produce interesting central effects, but these may in some cases be nonspecific and obtainable with the autonomically inactive degradation products (262).

**g Histamine and antihistaminics** Because of the possible role of vascular lesions in the etiology of convulsive and other central nervous disorders with EEG manifestations, and the presumed role of histamine in various inflammatory reactions, the effects of antihistaminics on the EEG are worthy of consideration. Churchill and Gammon (58) have observed that diphenhydramine can reduce the frequency of attacks in patients with petit mal, with a corresponding reduction in the incidence of spike and wave discharges in the EEG, whereas tripeleennamine has the opposite effect. In this connection it should be noted that diphenhydramine has been generally reported to have more sedative and other central nervous actions than tripeleennamine for equivalent antihistaminic effects. Swinyard (313) has observed that both drugs have some anticonvulsant effect on rats in doses which otherwise produce no gross neurological effects, and that both produce signs of excitation in the central nervous system when given in larger doses. However, tripeleennamine may cause the appearance of recurrent spontaneous seizures following an initial electroshock seizure, whereas this effect has not been seen with diphenhydramine. This differentiation between the two antihistaminics may have some relevance to the clinical observations of Churchill and Gammon. Histamine itself even in doses causing severe symptoms was without effect on the EEG of petit mal patients studied by Williams (343), in contrast to the sensitivity of these patients to acetylcholine and anticholinesterases.

In concluding this discussion on the EEG effects of autonomic drugs, the reviewers feel impelled to point out a remarkable contrast between the almost purely empirical data collated for these agents and the more analytical investigations which have been devoted to other drugs such as the barbiturates, for which plausible mechanisms of action could not so readily be assumed *a priori*. The result is that we have as yet no useful body of measurements on parameters of excitation and response against which to equate the observed EEG effects of these agents. Neither is there any body of knowledge which can be transposed from the periphery to the central nervous system to provide a working hypothesis for the interpretation of cortical electrical activity. Peripheral nerve cannot be used in this case to build a working model of the central nerve net, since the autonomic drugs, for example acetylcholine, are for the most part inactive on peripheral nerve (238). When they are effective in high concentrations, their actions may be attributable to side-effects which bear no relation to their *in vivo* specificity, as in the case of nerve conduction block by DFP (50, 52, 67, 281, 323). Even in the

case of neuroeffector junctions and autonomic ganglia, the attention of investigators has been occupied with the fact of chemical mediation rather than the intimate mechanism of activation or inhibition of the effector or post-synaptic cell. Since the central effects of autonomic agents cannot be inferred automatically from our present knowledge of peripheral fibers and synapses, it devolves upon the neuropharmacologist to approach the problem of the central action of a particular autonomic drug with as much variety of procedure, precaution and imagination as if the substance were new and unfamiliar.

In summary, the literature in general suggests that acetylcholine administered by various routes to man and animals may have excitant effects ranging from increasing frequency of EEG rhythms and facilitation of cortical excitation to seizure discharges in the EEG and frank motor convulsions. Depressant effects are occasionally reported, particularly with higher concentrations. These effects are potentiated by the prior administration of the common anticholinesterases, which by themselves may produce central responses not unlike those to acetylcholine, although certain discrepancies in their actions cast some doubt upon an identical and sole anticholinesterasic mechanism of action. Finally, the excitant or depressant effects of the cholinergic substances and anticholinesterases are usually found to be abolished by atropine in doses which alone may not significantly influence the normal EEG or produce neurological manifestations. Other anticholinergic drugs such as curare do not appear to have significant central effects comparable to atropine. These considerations should cast some suspicion on the concept that acetylcholine is the chemical mediator at central synapses rather than merely an auxiliary agent which acts indirectly, perhaps through its effect upon the vascular supply of the brain. Of other possible chemical mediators, neither histamine nor epinephrine has received enough attention to justify any conclusion regarding their role, if any, in central synaptic transmission.

2 *Metabolic agents* To what extent does the EEG reflect changes in cerebral metabolism? Throughout much of the literature on the electrical activity of the brain, there runs a strong thread of conviction that the frequency and amplitude of the recorded brain potentials are manifestations of the rates and magnitudes of underlying chemical processes, and that those drugs which modify the EEG do so by alteration of metabolic events (135). In a very general sense this is undoubtedly true, but the temptation is sometimes strong to oversimplify, for example, by considering cortical frequencies to be functions only of oxidative metabolic rates. That the situation must be more complicated is suggested by a brief consideration of the relation between function and metabolism in peripheral neurones as shown particularly by the work of Lorente de Nó (238). Oxygen is necessary for the maintenance of a membrane potential, which must be held above a critical value if excitation and propagation of impulses is to occur. However, the membrane potential may be experimentally restored by anodal polarization after nerves have been made non-conductive by anoxia or by metabolic blocking agents, and the nerve is then excitable and able to propagate impulses. Excitability is lost in the absence of sodium (and no other) ion even if membrane potential is maintained. No exogenous substance other than oxygen is required

for maintenance of membrane potential, since endogenous stores of metabolites may be drawn upon. Depolarization by anoxia or by inhibition of oxidative metabolism is not associated with changes in threshold until the critical point of conduction block is reached. Other aspects of function, such as the recovery process, are more sensitive to metabolic interference than those immediately concerned with impulse propagation. Carbon dioxide at physiological tensions maintains a greater membrane potential, a higher threshold and a great stability of the membrane against spontaneous firing or the effects of various excitant substances. Changes in environmental temperature produce complex effects which indicate that different temperature coefficients of underlying processes are concerned in several aspects of nerve function. Many substances act upon nerve in a manner not obviously related to oxidative metabolism.

The above are some of the metabolic factors which apply to relatively rugged large myelinated fibers. The situation is certainly more complicated for the more sensitive neurones of the central nervous system, with their higher metabolic rate and their requirement for exogenous metabolites. Therefore a simple and universal relationship between cerebral cortical function as represented by frequencies of EEG rhythms and the rates of underlying chemical processes should not be expected, and by the same token metabolic effects should not be assumed *a priori* for drugs which alter cortical rhythms.

Alteration in body temperature may be taken as one of the simple nonpharmacological methods for altering the rates of chemical processes in the brain. Hoagland (184, 185, 186, 187) found a simple linear relation between the logarithm of the frequency of the dominant EEG rhythm and the absolute temperature in patients undergoing diathermy treatment. The results conformed to the Arrhenius equation, and the calculated values of the critical thermal increment were found to be 8, 11 and 16 Calories, common values for steps in intermediate carbohydrate metabolism studied *in vitro*. The lower temperature coefficients were characteristic of normal subjects and the higher of advanced cases of general paresis. The results seemed to indicate that the alpha rhythm was a simple indication of metabolic rate determined by a limiting chemical pacemaker reaction, and to reveal changes in the limiting reaction in disease. However, Greenblatt and Rose (164) found complex changes in the EEG of patients with neurosyphilis treated with fever induced by typhoid vaccine or malaria. In the majority of these records alterations consisted of an increase in the irregularity of the pattern with increased amplitude and number of slow waves. A few fever records presented both rapid and slow activity, but with the slow activity dominating the pattern. Decline in fever resulted in the gradual resumption of the original characteristics. The authors found more marked changes in the EEG with a more rapid rise in temperature and with the higher final elevations of fever. In addition, the more severe the clinical picture of the disease the more marked were the changes elicited by fever therapy. Thus the manner of altering body temperature as well as the status of the patient enters into the EEG findings, and there seems to be no doubt but that the EEG can be grossly and qualitatively altered at the extreme tolerated limits of the temperature scale.

With this introductory note of caution, some of the EEG findings associated with gross metabolic changes, alterations in respiratory gas tensions and blood sugar, and the effects of metabolic blocking agents will now be considered

a **Thyroid** A correlation between the alpha frequency and the basal metabolic rate was found by Lindsley and Rubenstein (232), the administration of thyroxin to one subject over a period of three days increased the metabolic rate from 53.7 to 59.0 Calories per hour and increased the frequency of the alpha waves from 10.5–11.4 per second. Examination of the alpha rhythm in patients with various thyroid disorders has likewise revealed a direct relationship between basal metabolism and the rate of cortical discharge. Bertrand *et al* (23, 24) found a correlation between the alpha frequency and the basal metabolic rate in individuals with hypothyroidism, and Ross and Schwab (286), in 80 determinations of the dominant EEG frequency in a group of patients with thyroid disorders, found good correlation to exist within the whole group, which suggested a simple relation between the alpha rate and the metabolic state of the individual. Other authors (135, 181) have confirmed these observations in both hypo- and hyperthyroidism. Hoagland *et al* (187, 289) found that increase in the metabolic rate was associated with a rise in alpha frequency following the administration of large doses of thyroxin intravenously for a period of 4 weeks.

b **Dinitrophenol** Dinitrophenol, a specific metabolic stimulant, has likewise been observed by Hoagland *et al* (182, 191) to cause an increase in the alpha frequency, the progressive and continuous acceleration tending to confirm the view that the frequencies are a measure of cortical respiration under these conditions.

c **Anoxia** The effect of oxygen lack on the human EEG has usually been reported as a slowing of frequency, and has often been taken to indicate a direct relation between oxygen uptake and dominant EEG frequency. The literature has been reviewed by Brazier (31, 33). Her own investigations (33) indicate that with increasing depths of anoxia there is a progressive slowing of the alpha rhythm, suggestive of deceleration of the synchronized beat of a uniform neurone population, as long as consciousness is retained. With the onset of unconsciousness there is an abrupt appearance of slow activity, suggesting the disorganization or depression of cortical rhythms in such a way as to release slow rhythms originating from subcortical structures. These findings agree in a general way with those of previous investigators, although a number of variations on this theme have been reported. Berger (22) was impressed more by the irregularity of the alpha rhythm than by any change in frequency prior to the onset of slow activity during anoxia, and Lindsley and Rubenstein (232) also failed to find any consistent change in frequency or amplitude of alpha rhythm during moderate anoxia.

Davis *et al* (82) observed the following more detailed sequence in subjects breathing 8 to 11 per cent oxygen mixtures: at first there was a slight increase in average voltage, with the appearance of alpha waves in those records which had originally shown none, then the alpha voltage decreased, the alpha trains were reduced in duration and the intervals between them became longer, 7 to 8 per

second activity began at the vertex while 10 per second alpha continued at the occiput, with the development of slight cyanosis and the first subjective changes, irregular slow waves appeared at the vertex and almost immediately thereafter at the occiput, alternating with a 10 per second rhythm, finally, after 10 to 15 minutes of hypoxia, slow waves dominated the record, and consciousness was definitely lost in some subjects. An abrupt transition from slow waves to alpha rhythm occurred promptly on restoration to room air. Thus the records of Davis *et al* are more indicative of qualitative transitions than of progressive continuous slowing of frequency. Gibbs *et al* (133, 140, 142), who observed that alpha rhythm was maintained at relatively normal frequency up to the point of unconsciousness during anoxia provided that the  $p\text{CO}_2$  of the internal jugular blood was maintained at normal levels, have pointed to the role of cerebral apnea during hypoxic overventilation as a factor in the slowing of cerebral rhythms.

Among those who have emphasized a progressive slowing of frequency during anoxia are Hoagland (187) and Engel *et al* (97, 100), using manual methods of frequency analysis, and Brazier *et al* (32, 33, 109), using the Walter method of frequency analysis (330). It is interesting to note that the shifts in frequency reported by this latter group are not very great and are increased by a fall in  $p\text{CO}_2$ . The alternative method of frequency analysis devised by Grass and Gibbs (160) shows a maintenance of frequency of the dominant alpha peak with some reduction in height of this component, associated with an increase in the energy distributed through lower frequency bands.

Thus the controversy concerning the effects of anoxia on the human EEG is based to a considerable extent on the method of interpretation of frequencies and will probably not be resolved until single unit discharges can be analyzed experimentally. Meanwhile the conclusion may be drawn that there is an average slowing of frequency based in part on a small change in frequency of particular components and in part on the entry of new slower components in the composite record. The variations in results reported by different investigators may depend in part on the method of frequency analysis, the area from which recording was taken, and the degree of hypocapnia associated with hypoxic overventilation, which is notoriously variable in man.

More severe degrees of anoxia than are feasible in man have been studied in experimental animals. Bremer and Thomas (44) observed a period of sleep-like activity in the EEG of the midbrain-transected cat preceding complete suppression of the EEG by asphyxiation. Sugar and Gerard (309) recorded the electrical activity of the cortex and various subcortical structures of the cat during and following a period of ischemia of the brain. In the motor cortex they observed an early increase in frequency and amplitude of the dominant activity of the EEG, associated with such motor signs as hyperpnea and convulsive movements. The fast activity was replaced by 1-3 per second slow activity before complete suppression of the EEG. On restoration of the blood supply, slow waves reappeared first, followed by bursts of unusual spindle activity of 6-9 per second and high amplitude, preceding the return of normal fast activity. A similar sequence was seen qualitatively in various subcortical regions, the functionally lower and less

complex centers requiring a longer period of ischemia. Throughout the brain fast activity was more susceptible than slow waves to ischemia, except for the initial transient increase in fast waves noted early in the records. Increased synchronization of various brain areas was noted during moderate ischemia.

Gellhorn and Heymans (123) compared the effects of anoxia and asphyxia on the EEG of dogs and cats. Simple anoxia produced by ventilation with low oxygen gas mixtures failed to produce the initial increase in fast activity noted with asphyxiation. Convulsive spikes evoked by strychnine were more vulnerable to suppression by anoxia or asphyxia than was the prevailing background activity. Since carbon dioxide alone produced increased frequency and amplitude of both normal and convulsive potentials, it is evident that the initial acceleration of activity during asphyxia should not be attributed to oxygen lack (309). A "rebound" phase of increased excitability and electrical activity was seen during the recovery period. Gellhorn (120) has also presented evidence that under conditions of asphyxia and anoxia the hypothalamus and thalamus may act as pacemakers of cortical activity.

Although Sugar and Gerard (309) had found that slow activity was more resistant than fast potentials to asphyxia, this relation does not appear to hold for recovery after prolonged asphyxia. Van Harreveld (173) found that after 15 minutes or less of asphyxiation, bursts of 7-12 per second activity similar to those found in sleep were the characteristic feature of the EEG of the cat. They were considered to be of cortical origin because of their asynchrony in various EEG leads, but the possibility of a mosaic subcortical origin should also have been considered. After more than 15 minutes of asphyxial suppression of the EEG, the typical activity on recovery consisted of smooth sinusoidal spindles of 12-16 per second frequency, synchronous throughout the cortex and presumably of subcortical origin.

As an illustration that neurones at all levels of the nervous system do not behave identically, attention should be called to the research of Brooks and Eccles (48) on the effects of asphyxia and anoxia on monosynaptic transmission in the spinal cord of the cat. Their results are the inverse of those of Gellhorn on the cerebral cortex. They found that anoxia produced a phase of hyperexcitability associated with progressive depolarization until the critical point for synaptic failure was reached. A period of depression followed the readmission of oxygen. Asphyxia was similar in effect except for a transient initial depression, and carbon dioxide had a purely depressant action. The direct effects of anoxia on peripheral nerve, as observed by Wright (347) and Lorente de N6 (238), do not seem to include marked changes in threshold until the degree of depolarization becomes critical.

d Hyperoxia. Detailed reviews of the effects of excessive oxygen on the nervous system have been published by Stadie *et al* (303) and Bean (12). In laboratory animals, convulsive activity is a characteristic feature of the effect of oxygen pressures considerably in excess of one atmosphere. EEG observations on the effects of compression are largely limited to the work of Cohn and Gersh (63) who studied the effects of oxygen poisoning in cats confined in pressure tanks.

With oxygen at atmospheric pressure, the records were essentially unchanged from those observed in room air. After one minute under a pressure of 8 atmospheres, high voltage slow discharges appeared, superimposed on the 20 per second activity observed under control conditions. The convulsive phase began with an increase in the number of high voltage slow waves, then high voltage 15-18 per second discharges appeared, and finally after 6 minutes a typical seizure pattern occurred and was repeated for several minutes. The seizures could be prevented by pretreatment with anticonvulsant drugs (270). The investigators considered the ultimate cause of seizures during oxygen poisoning to be damage to the metabolic system of nerve cells, perhaps to enzymes having SH groups, and to a consequent reduction in threshold. Since oxygen poisoning is apparently associated with increases in blood and brain glucose,  $pO_2$ ,  $pCO_2$ , and acidity, all of which militate against the appearance of slow waves in the EEG, the early appearance of slow activity was attributed to some basic intracellular change rather than to the gross secondary alterations in the internal environment. The specificity of oxygen excess for particular enzyme systems is still to be worked out in relation to the observed neurological syndrome of oxygen poisoning.

e Carbon dioxide. It has long been recognized that changes in  $CO_2$  tension have important effects upon central nervous function, and those alterations produced by hyperventilation have been of particular interest to the electroencephalographer because of the ease with which paroxysmal dysrhythmias can be precipitated by overbreathing in some types of convulsive disorder. The subject has been reviewed by Brazier (31) and will be dealt only a glancing blow in the present discussion.

Foerster (111) is usually given credit for the demonstration that epileptic attacks can sometimes be elicited by hyperventilation, and before the advent of electroencephalography this diagnostic method had already been used critically by Lennox (219) in petit mal. In one of his classical studies, Berger (22) elicited high voltage slow wave discharges preceding a major seizure in one patient with a previous history of attacks. It has gradually been established that seizures of the petit mal triad rather than convulsive disorders in general are sensitive to small changes in  $CO_2$  tension (139, 218, 219, 221, 224), however, other types of attack or EEG abnormalities may occasionally be induced by prolonged hyperventilation (46, 282), and occasional atypical forms of petit mal are resistant to hyperventilation or  $CO_2$  excess in spite of the presence of a typical 3 per second spike and wave dysrhythmia (317).

The effects of overventilation on the normal EEG are usually reported as slowing of frequency, with the appearance of high voltage 3-6 per second slow wave discharges particularly in the frontal leads in some patients. In the experience of the reviewers, these paroxysms are found in about 10 per cent of healthy male medical students. They are more easily elicited in infants and children, decline in incidence with advancing age (139, 229), are easier to invoke at low blood sugar levels (36, 81, 180, 294, 307), and are subject to modification by anoxia and other metabolic variables (76, 77, 94, 97, 122). The dependence of hyperventilation-induced changes in the EEG upon cerebral blood flow has been established by



Gibbs *et al* (129, 140, 225) who recorded the EEG responses during overbreathing or CO<sub>2</sub> inhalation while making simultaneous determinations of CO<sub>2</sub> content, pH, CO<sub>2</sub> tension and oxygen saturation of arterial and venous blood. The dilatation or constriction of cerebral arterioles which follows an increase or decrease of CO<sub>2</sub> in arterial blood normally serves to protect the brain against an undue shift of CO<sub>2</sub> tension. The slow waves that appear in the EEG with hyperventilation are concluded by these investigators to be caused by a drop in CO<sub>2</sub>, not by anoxia secondary to cerebral vasoconstriction as had been previously postulated (76, 77, 129). The ease with which slow waves can be evoked by hyperventilation is a rough index of the relative incompetence of the cerebral vasoconstrictor response to a low CO<sub>2</sub> tension.

The effects of inhalation of CO<sub>2</sub> on the human EEG are usually described as an increase in frequency of cortical potentials (126, 142, 215, 224). The experimental literature does not show good agreement as to the EEG and other central nervous effects of changes in CO<sub>2</sub> tension. An excitatory effect of CO<sub>2</sub> has been described, evidenced by an increase in frequency both of background activity and of convulsive potentials (123). However, some investigators have reported a protective effect of carbon dioxide inhalation against metrazol (125) and strychnine (209) seizures, whereas others (179, 201) have found that convulsive metrazol discharges are singularly unresponsive to hyperventilation, CO<sub>2</sub> inhalation, and intravenous injection of acid or alkaline solutions. As for the normal EEG of animals, it has been reported that metabolic or respiratory alkalosis increases spontaneous cortical activity and decreases the threshold for afterdischarge while acidosis has the opposite effects (92). But others have found that severe acid-base changes are required to produce EEG abnormalities (240) or changes in seizure threshold (179).

To the extent that conclusions can be drawn from these observations, it seems clear that alterations in the EEG or in excitability are relatively difficult to evoke by acid-base changes, are more related to pCO<sub>2</sub> than to corresponding pH changes effected by other procedures, and are in the direction of increased EEG frequency and amplitude and decreased electrical threshold when the pCO<sub>2</sub> is reduced. These changes are what might be expected from the behavior of peripheral neurones, which show increased excitability to low CO<sub>2</sub> (213), dependent upon the pCO<sub>2</sub> itself rather than the induced pH change (238). Although increased pCO<sub>2</sub> in the absence of pH changes tends to raise threshold and prevent spontaneous firing in nerve (238), there are associated changes, such as increased amplitude of action potentials and after-potentials with associated changes in the post-firing recovery of excitability, which might complicate the behavior of a neural network. The sensitivity of various central neurones to acid-base changes varies widely (208). As an example of the extreme contrast to be found in the central nervous system, CO<sub>2</sub> has a purely depressant action on spinal motor-neurones (48), but it increases discharges from the inspiratory center of the medulla (304).

If one important lesson can be drawn from the literature on the effects of CO<sub>2</sub> on the EEG, it is that the petit mal 3 per second spike and wave discharges are

far more sensitive to  $p\text{CO}_2$  than is any other type of cortical electrical activity. While for a long time there was a tendency to ascribe petit mal to diffuse changes in cerebral function, there has been a recent revival of the Jacksonian principle that different seizure types arise from the different anatomical foci, and this concept has led to a clinical and experimental search for a subcortical origin of petit mal discharges (200). If such centers can be identified with certainty, it would be most interesting to determine whether they show unusual sensitivity to changes in  $p\text{CO}_2$ , and whether this is an attribute of the neurones as such or a result of peculiarities of their vascular supply.

**f Ketogenic diet** Although numerous clinical reports of the antiepileptic effects of the ketogenic diet have been published, few data concerning its effect on the EEG have appeared. Logan and Baldes (236) reported that there was a good correlation between clinical and EEG improvement in a series of 10 patients with convulsive disorders treated by ketogenic diet. Hoffman (196) has obtained records from 20 epileptic children with spike-wave dysrhythmias before and during their preliminary fast and at intervals thereafter while they were receiving a ketogenic diet. During the third, fourth and fifth days of fasting and at the time when hypoglycemia, ketosis and acidosis were most pronounced, EEG frequencies became slower, with a high voltage 3 per second rhythm predominating. As this phase gave way to compensated ketosis, frequency and amplitude returned toward normal, and a more prolonged period of hyperventilation was required to bring out the characteristic spike and wave picture than in the control period. When the diet successfully controlled seizures over a long period, the paroxysmal dysrhythmia disappeared from the resting record and could not be restored by hyperventilation.

The reviewers (317) have looked unsuccessfully for changes in the properties of peripheral nerve after immersion in high concentrations of several substances which have been considered by various authors as the effective therapeutic agents resulting from a ketogenic diet, including glycerol, beta-hydroxy butyric acid, acetone and acetoacetic acid.

**g Blood sugar** Interest in the EEG effects of insulin and blood sugar changes has been heightened by the wide use of insulin hypoglycemia in the shock treatment of the major psychoses. Numerous investigators (9, 35, 80, 81, 100, 145, 188, 190, 192, 215) have observed progressive slowing of the dominant frequencies associated with lowering of the blood sugar level, followed by the abrupt appearance of very slow waves as the patient lapsed into coma.

A direct relationship has been found by Himwich, Hoagland, and their colleagues between the frequency of the alpha waves and the cerebral oxygen consumption, both decline during hypoglycemia and increase after the administration of carbohydrate. Conversely, the delta index, a measure of the percentage time and amplitude of irregular high voltage slow waves, displays an inverse relationship to the blood sugar concentration and the oxygen utilization of the brain. These relationships are thought to indicate that the slowing of the EEG depends upon a progressive decrease in cerebral metabolism when the concentration of the principal substrate is reduced (182, 183, 188).

In contrast to the effects of hypoglycemia, Brazier *et al* (35) have found that above 130 mgm per cent further elevation of the blood sugar fails to alter the EEG of the normal adult. Abnormal records may be more sensitive to hyperglycemia, since Lennox and the Gibbs (137, 138, 224) have demonstrated that elevation of the blood sugar concentration tends to prevent the 3 per second spike and wave discharges of petit mal. This finding is of some practical importance in the establishment of relatively basal conditions for EEG recording. Failure to take into account the postprandial biphasic variation in blood sugar may be a source of uncontrolled variability in the incidence of EEG abnormalities and may give a false impression of the effectiveness of drug or other therapy.

The tendency for a low blood sugar level to increase the sensitivity to hyperventilation has been reported by several investigators (36, 180, 294, 307). Gibbs, Gibbs and Lennox (137, 138) have pointed out that abnormal discharges which were affected by variations in the blood sugar level (particularly the 3 per second spike and wave of petit mal) also generally responded to variation in the CO<sub>2</sub> and vice versa.

A summation of the central effects of oxygen lack and hypoglycemia has been demonstrated clinically and experimentally by Gellhorn *et al* (119, 124). For example, in rats and cats whose blood sugar level had been reduced by insulin to the point where slow activity dominated the EEG, the administration of pure oxygen could restore the normal activity, whereas inhalation of 7 to 8% oxygen mixtures, which normally had no EEG effect, could abolish the electrical activity of the cortex entirely. Sugar and Gerard (309) observed that insulin hyperglycemia hastened the EEG failure produced by cerebral ischemia in the brain of the cat.

Maddock *et al* (241) have studied the effects of hypoglycemia produced by hepatectomy and evisceration of various laboratory animals, and found the EEG changes essentially similar to those produced by insulin. Utilizing this method to determine whether other substances could substitute for glucose in brain metabolism, they found that the EEG effects could be reversed by the intravenous administration of glucose, mannose and maltose, but not by fructose, galactose, hexose diphosphate (with or without adenylic acid), glyceric aldehyde, succinate, fumarate, pyruvate, glutamate, or mixtures of glyceric aldehyde and glutamate or of glutamate and pyruvate.

In addition to the acute effects of low blood sugar, the persistent EEG sequelae of repeated bouts of hypoglycemia have also been investigated. Knott and Gottlieb (206) concluded that the outstanding effect of a course of insulin shock treatments on EEGs taken one to 12 days after completion of a series of treatments was an increase in the alpha index, most prominent in the frontal areas. Some patients showed an increase of activity below 6 per second. Greenblatt *et al* (163) found an incidence of more than 50 per cent abnormal EEGs during long-term observations of the effects of hypoglycemia on a group of diabetics who had suffered repeated insulin reactions.

To summarize, the effects of insulin hypoglycemia on the EEG include some degree of slowing of the dominant frequency and a sharp transition to slower

activity with the onset of coma, these changes are potentiated by anoxia and low  $\text{CO}_2$  tension. They are presumably related to a limitation of cerebral metabolism by restriction of the principal substrate, but the actual neural mechanism of slowing has not been experimentally defined.

**h Cyanide** The use of cyanide in the determination of circulation time man has presented an opportunity for the study of the EEG effects of this. After intravenous injection, the arrival of cyanide in the arterial circulation signalled by a brief hyperpnea, probably of carotid body origin, and EEG slowing can be detected shortly thereafter. Rubin and Freeman (290) usually observe an increase in the amplitude, regularity and amount of alpha activity following the intravenous administration of 0.7 cc of two per cent sodium cyanide solution. In a few instances, the increase of alpha activity was followed by a decrease. In addition, higher frequencies became more predominant and slow, irregular rhythms tended to disappear, while the frequency remained unaltered. An initial slight decrease of alpha activity was observed in a few cases, in some instances, it remained unchanged. In two cases however, bursts of high voltage regular slow activity were seen. This is more in line with the experience of Lipton and Gibbs (233), who also recorded an initial increase in activity in the alpha range following rapid intravenous injection of unspecified small doses, but in addition saw high voltage 2-3 per second waves at the peak of drug action. With still higher doses, very slow activity at  $\frac{1}{2}$ -1 per second appeared, with a decrease in amplitude which sometimes proceeded to complete flattening of the record. When cyanide was given rapidly in sufficiently high dosage, sudden flattening occurred without preceding slowing.

In cats given a sufficiently large dose of cyanide, an interesting form of decerebrate rigidity occurs, often irreversibly. Ward and Wheatley (33) have investigated this phenomenon and found that it is based on swelling of the diencephalon secondary to circulatory changes and severe brain edema produced by the substance.

Since the cyanide ion is primarily an inhibitor of oxidative metabolism, it might be expected that the effects in all systems would be identical with those of anoxia. The accentuation of alpha rhythm described above in human patients indicates that the effects are not identical. Although the difference might be ascribed to the extra-cerebral (e.g., carotid body) effects, Lorente de N6 (238) in his studies on peripheral nerve has observed an interesting action of cyanide in increasing the negative after potential. This and alterations in other properties of neurones indicate that cyanide, unlike anoxia, produces a relative increase in the labile fraction of the membrane potential even while typical anoxic depolarization is progressing. In other respects the ion acts simply as if it were an inhibitor of oxygen uptake.

**i Fluoroacetate** The rodenticide fluoroacetate offers a minor public health problem in that it is an occasional source of epileptiform seizures after accidental ingestion by man. The physiology, pharmacology and biochemistry of this substance have recently been reviewed in the most definitive fashion by Chenoweth (54), who has also made extensive EEG studies (56, 57). Fluoroacetate blocks

a number of metabolic reactions involving the acetate ion, but the ultimate manifestations of this common action vary widely from species to species, depending apparently on quantitative differences in various tissues in the effects of the disruption of intermediate carbohydrate metabolism (54, 55) In man, fluoroacetate poisoning may be manifested by epileptiform seizures, but death may result from cardiac failure as a result of ventricular fibrillation Some species, such as dogs and rats, show predominantly central nervous effects, including convulsions and respiratory failure Other species, such as the rabbit, show primary cardiac failure Man and rhesus monkey are intermediate in response

Chenoweth and St John (56, 57) recorded the EEG of curarized dogs following the intravenous administration of fluoroacetate The first changes observed were increases in frequency and amplitude of temporoparietal and occipital activity while frontal and cerebellar potentials remained unaffected Two types of seizure patterns were observed in the EEG a high frequency high voltage discharge of the kind commonly seen during major convulsions in human or animals, and a spike and wave type of discharge sometimes resembling superficially that of the paroxysmal dysrhythmia of petit mal in man Chenoweth *et al* have stressed this resemblance to clinical petit mal, but their records seem to indicate that the spike-wave discharges may be of many forms and may occur over a wide frequency range Convulsive discharges in the EEG and convulsive motor manifestations could be prevented by treatment with barbiturates, anticonvulsants and carbon dioxide excess

Ward (332) has recorded simultaneously the electrical activity of the cortex and subcortical structures following intravenous administration of fluoroacetate in cats After a latent period of several hours characterized only by depression, seizure discharges began in the cortex, thalamus and hypothalamus, associated with motor manifestations Hypothalamic slow activity occurred independently of that of the cortex, which showed waves or spike and wave formations of 3 to 8 per second, while the thalamic records were characterized by spikes of high frequency and low amplitude The three centers were unsynchronized except that the thalamic spikes were modulated in synchrony with the cortical waves

Among interesting findings related to fluoroacetate seizures, Chenoweth (54) points out that animals such as rabbits which do not convulse after intravenous administration of fluoroacetate in any dosage may be made to do so by intracranial injection Fluoroacetate seizures are preceded by a progressive decrease in the threshold for electroshock convulsions They are potentiated by neostigmine, which suggests but does not prove a role of acetylcholine in their production The substance is not specific for higher centers, since local application to the cord or systemic administration after spinal transection may produce cord seizures following a period of accentuation of all spinal reflexes Peripheral nerve treated with methyl fluoroacetate does not exhibit increased excitability, conduction is ultimately blocked following an increase in threshold

The metabolic correlates of these actions have also been reviewed by Chenoweth (54) Gerard and his students (50a, 128) have shown that in frog brain the amplitude of the electrical activity and the oxygen consumption both may be re-

duced to about 50 per cent of normal by saturating doses of methyl fluoroacetate. However, the lack of enzymatic specificity is seen in the depression of activity of other enzymes such as cholinesterase. Of various carbohydrate intermediates, fumarate is most effective in preventing the effects of fluoroacetate. In frog nerve the resting oxygen uptake is depressed while the active rise during propagation of impulses is unaffected (30a, 89).

Chenoweth (54) emphasizes the parallelism in resistance of various species and tissues to fluoroacetate, anoxia and cyanide, based presumably on the importance of oxidative as against glycolytic metabolism in the maintenance of their function. The specific metabolic blocking action of fluoroacetate is apparently based on its close structural similarity to acetate ion, preventing both complete oxidation of acetate and its entry into the Krebs cycle. Thus, *in vitro* studies have shown that acetate accumulates from a pyruvate substrate with a fall in oxygen uptake, and the formation of succinate is blocked. Acetoacetate may still be formed from acetate but not vice versa,—apparently a mass action effect. Various foreign amines may be acetylated in the presence of the excess acetate, but acetylcholine formation is not altered.

In summary, the structural blocking action of fluoroacetate may result in convulsive manifestations and associated EEG changes, apparently in part through an increase in excitability which is seen throughout the central nervous system but not in peripheral nerve, although oxygen uptake of both brain and nerve is depressed. Obviously these effects are quite different from those of simple anoxia. Skillful use of such pharmacological tools as fluoroacetate brings promise of the early unraveling of many problems in the relation of specific enzyme systems to nervous function. It should of course be kept in mind that non-specific side effects of blocking agents are the rule rather than the exception, and the specificity requires proof in each new experimental situation.

*3 Miscellaneous agents and procedures* There are many casual reports in the literature concerning the EEG effects of substances which have not been further investigated in regard to their mechanism of action, either because of their apparent lack of theoretical or practical interest or because of the peculiar tenacity with which neuropharmacological research clings to comfortable channels until social urgency and new financial sources of aid to research cause the stream to overflow. Conversely, many substances for which other effects upon the central nervous system or upon peripheral nerve have been well described have failed to appear in the schedule of the busy electroencephalographer, sometimes even when their clinical usage is quite general. The resulting lack of tangency between empirical EEG effects and other neurological actions has been the subject of previous complaint in this review, and has discouraged the reviewers from any attempt to be complete and systematic until the field has matured considerably. Therefore, the remainder of this discussion will be devoted to a consideration of a few agents which have sufficient clinical or theoretical interest to merit further research on the mechanism of their action upon the EEG.

*a Water* The effects of hydration on the EEG have received attention only recently, although pitressin antidiuresis and forced water ingestion have been

used for many years to precipitate seizures for diagnostic purposes (250), and dehydration has long been known to have some therapeutic value in convulsive disorders (104). Wikler (342) recorded the EEG of 14 male patients who had no history or clinical evidence of epilepsy. After injection of pitressin and an intake of water sufficient to raise the body weight an average of three kg, half of the cases showed paroxysmal slow activity. This change was poorly correlated with the degree of hydration. No seizures were produced in this control series. The alpha frequency showed a tendency to slowing, but in only three instances was the degree of change greater than that expected from day to day variation. There was no significant change in the percentage time alpha activity. Cohn, Kolb and Mulder (65) attempted to validate the pitressin-water test in a group of 23 men, some of whom showed clinical and EEG evidence of a convulsive disorder, while the control series had no manifestations that supported the diagnosis of epilepsy. Of the 23 men, 20 showed a progressive increase in slow activity in the EEG regardless of previous history, of five patients who developed convulsive seizures, two manifested no preconvulsive changes during the procedure. Blier and Redlich (27) obtained somewhat more positive results with the pitressin-water test in three groups of patients with initially normal EEG records. After hydration, three of the 11 patients with a definite history of convulsive seizures revealed paroxysmal EEG changes, including fast activity, petit mal bursts, and "psychomotor" sharp waves, six showed minor alterations, as did one of 11 patients with a questionable diagnosis and one out of 10 controls. Kaufmann *et al* (204) attempted hydration as an activation technic in a group of post-traumatic epileptics and a group of control post-traumatic encephalopathies without seizures. No patient showed EEG changes, although one epileptic had a generalized seizure two hours after the test. Summarizing these clinical observations, it can be said that the EEG changes observed during hydration are not highly specific for convulsive disorders.

Water intoxication in rats has been found by Gellhorn and Ballin (121) to produce slow waves of 1-3 per second frequency and high amplitude, appearing singly or in groups. When the water dosage was lethal, these waves gradually declined in amplitude until the animals died in deep coma as cortical activity disappeared. Following the onset of slow waves, convulsive discharges also were seen in some animals either as single or multiple spikes or in combination with slow waves, occurring either as larval discharges or associated with convulsions. In animals treated with desoxycorticosterone, the EEG changes consisted largely of slow waves only, without convulsive discharges.

Pick and Miller (272) found that loss of diffusible electrolyte in frogs, accomplished by keeping them in distilled water for one to four weeks, caused a marked decrease in frequency and amplitude of brain potentials.

Both animal and clinical investigations therefore seem to indicate that water intoxication can cause the appearance of slow waves if the hydration is severe enough, and that more vigorous dilution of the body fluids sometimes but not invariably may elicit convulsive discharges.

There have been no systematic studies of the effects of hydration at all levels

of the nervous system, but it is known that cortical threshold for electrically or chemically induced seizures are dramatically lowered by hydration (314)

b Antibiotics The occasional occurrence of seizures following the therapeutic intraventricular use of penicillin has led to a number of studies of the EEG effect of this and other antibiotics, chiefly by Walker and his colleagues (29, 202, 203, 204, 308, 326, 328, 329), who have also reviewed the literature in the field (327, 328) Seizures and EEG changes are not seen when even large doses are given intravenously or intrathecally, but may appear when the antibiotics have direct access to the brain by intracisternal, intraventricular or subdural administration Subconvulsive EEG changes in man following intraventricular penicillin include slowing of the alpha rhythm, increased amplitudes and the appearance of fast spike activity Preconvulsive single and multiple spikes have been described in cats and monkeys after local application of penicillin to the cortex (29, 326), and a similar picture is reported for streptomycin in various species (308) Fortunately, there seems to be a wide margin of safety between antibiotic concentration and convulsive threshold for both penicillin and streptomycin, so that *their use in cerebral infections is not dangerous if the final concentration in the cerebrospinal fluid is properly controlled*, however, the margin is less for streptothricin, actinomycin and clavacin (203, 328)

As to the mechanism of convulsive action of the antibiotics, little can be deduced from the literature The reviewers (317) have noted that streptomycin is unlike the typical convulsants in that it produces only evidence of depression when administered intracisternally or intrathecally in frogs High concentrations appear to block conduction in frog peripheral nerve by a process of depolarization

c Sulfonamides The sulfonamides are also capable of producing convulsive manifestations upon local application to the cerebral cortex Epileptic seizures have been observed following topical application of sulfathiazole to the brain of man (334) and of experimental animals (274) Jasper *et al* (199) have studied the effects of microcrystalline sulfonamides upon the EEG of the monkey With sulfathiazole this vigorous treatment of the cortex resulted in the appearance of a variety of persistent dysrhythmias, with preconvulsive spikes or sharp waves and focal seizure activity as frequent findings Sulfapyridine produced only depression of activity and sulfanilamide and sulfadiazine were without effect Aside from osmotic and other factors which may facilitate seizures when sulfonamides are applied topically, sulfathiazole may have convulsant effects when given systemically (199) The mechanism has not been studied

d Antimalarials Because the EEG effect of antimalarials in routine clinical practice may be complicated by fever and cerebral involvement, the observations of Engel *et al* (96) on normal control subjects receiving quinacrine are of some interest These authors report a progressive and sustained increase in average frequency of cortical rhythms, associated with signs of restlessness and tension The supposed anticholinesterasic activity of quinacrine is invoked by Engel *et al* to explain the clinical and EEG findings In contrast, Pick and Hunter (271) conclude that quinacrine has a depressant effect on cortical activity of cats



and frogs Gallouin and Lemaire (117) have noted a reduction in amplitude of alpha rhythm in patients receiving single doses of quinine. Perhaps more systematic examination of the antimalarials will be inspired by the growing realization that these and other chemotherapeutic agents may have highly specific effects upon particular enzyme systems.

e Steroid hormones. Passing mention will be made of progesterone and desovycorticosterone acetate (DOCA) only because these steroids have frequently been reported to have central depressant effects in large doses (cf 297). Engel and Romano (95) found that DOCA could partially restore the abnormally slow EEG in patients in Addisonian crisis. Clinical anticonvulsant effects have been claimed for chronic DOCA administration (251), and adrenal cortical extracts have been reported to improve both clinical and EEG signs of post-concussion syndrome (4) and to protect the exposed brains of animals from edema and associated slowing and reduction in amplitude of the EEG, presumably by a stabilizing action upon blood vessels (165, 166). Total adrenal cortical extract is said to increase the frequency of the dominant EEG rhythm in man, in contrast to a slowing effect of DOCA (167). The central effects of the adrenal cortical steroid hormones could perhaps be better evaluated if a greater effort were made to differentiate experimentally their concomitant actions upon water and electrolyte balance.

Progesterone (with diethyl stilbestrol) in doses sufficient to induce menstruation in menopausal women is said to be without effect upon the EEG (68).

f Agenized flour products. Recent investigations on "canine hysteria", a convulsive disorder in dogs maintained upon a white flour diet, have roused considerable apprehension regarding the possibility of related central nervous dysfunctions in man. The syndrome was identified with the wheat gluten fraction by Wagner and Elvehjem (325), and was shown by Mellanby (252) to result from the "agene" process utilizing nitrogen chloride as a bleaching agent. The active principle was finally localized by Silver *et al* (298) to agenization products of cysteine and cystine. The EEG correlates of the syndrome in dogs were studied by Erickson *et al* (101, 260, 261), who recorded preconvulsive 2-3 per second waves of high voltage and typical tonic-clonic seizure discharges during the attacks. Silver (298) observed convulsive dysrhythmias in cats and dogs, and occasional slow abnormalities in monkeys, which ordinarily develop asynergy, tremor and weakness rather than seizures; these investigators established the ominous fact that the disorder once initiated was relatively irreversible. Newell *et al* (261) failed to find EEG or clinical changes in patients given a diet rich in agenized products for periods of several weeks, but the invulnerability of man to this civilized dietary refinement has not yet been proved beyond a doubt.

#### V CONCLUSION

From the foregoing review it should by now be obvious that the actions of drugs upon the EEG are at best poorly understood, even though the literature is replete with empirical observations of chemically induced alterations in the electrical activity of the brain. Only a limited number of drugs,—chiefly strychnine,

ether, the barbiturates, and some of the antiepileptics effective in grand mal,—have been examined with sufficient thoroughness to permit a tentative interpretation of their observed effects on the EEG in the light of their ability to bring about particular changes in the neurones of the brain

Our prevailing ignorance of the mechanisms of action of most drugs upon the EEG should therefore serve as a warning against any easy classification of drug effects as depressant, excitant, etc., on the basis of their modification of the EEG. For example, to interpret an increase in frequency of cortical waves as evidence of an excitant pharmacological effect is to ignore the many other mechanisms by which even depressant substances such as the barbiturates may cause an apparent increase in EEG frequencies at some particular dose level or stage of action. Here as in other fields the passive acceptance of oversimplified generalizations can lead to the disorientation of further investigation and delay progress both in research and in therapy.

Another source of error in the interpretation of the EEG is the assumption that any observed alterations are the result of the direct action of the drug upon the cortex itself, when in the particular case the effects may be secondary to more fundamental actions elsewhere in the body. Thus curariform drugs were thought to have profound direct EEG manifestations at one time until the role of anoxia secondary to neuromuscular paralysis was taken into account.

Finally it should be remarked that the action of drugs on the normal EEG must be sharply distinguished from those manifested in the presence of preexisting abnormalities, particularly discharges of convulsive type. Since convulsive discharges seem to arise in a way qualitatively different from the normal components of the EEG and may be altered or obliterated by a number of different mechanisms, erroneous conclusions may easily be drawn in attempting to translate evidence from one category to the other.

With respect to future work, it becomes clear that many refined and thorough neurophysiological investigations will be needed to close the gap between our present empirical information and our theoretical insight into mechanisms of drug action upon the EEG. Such analysis will undoubtedly necessitate the development of new methods for measuring the excitable and response characteristics of cerebral neurones, preferably of single cells. Meanwhile the empirical clinical literature would benefit from more careful description of the character of drug-induced changes in the EEG as well as the circumstances under which they were elicited, in preference to the mere classification of EEG changes. Similarly it is important to record other observed or known effects of the drugs used, in order to be able to recognize secondary effects upon the EEG when they occur.

In conclusion, the EEG taken alone may often give frivolous or misleading information concerning the nature of drug action, but when supplemented with information obtained by other methods it can add materially to our knowledge of the pharmacological actions of those drugs which have demonstrable central nervous effects. Conversely, a more systematic pursuit of the EEG correlates of drugs with well-known specific effects upon properties of neurones or upon

enzyme systems would in turn probably lead to a better understanding of the nature of the recorded electrical activity of the brain

#### ACKNOWLEDGEMENT

The authors are deeply indebted to Dr Louis S Goodman for valuable advice and criticism, and to Miss Lola Madge Henrie for bibliographic assistance

#### REFERENCES

- 1 ADRIAN, E. D. The spread of activity in the cerebral cortex. *J. Physiol.*, 88: 127-161, 1933.
- 2 ADRIAN, E. D. *The Physical Background of Perception*. Oxford University, Oxford, England, 1947
- 3 ADRIAN, E. D. AND MORITZ, G. Impulses in the pyramidal tract. *J. Physiol.*, 97: 153-199, 1939
- 4 AIRD, R. B., STRAIT, L., BRENNER, M., AND ZEOLKE, D. Neurophysiological studies on cerebral concussion. *Prog. Am. Neurol. Assoc.* 22-23, 1947
- 5 ALMONTE MARSAN AND FUORTES, M. G. F. Electrographic study of the convulsant action of intravenously administered acetylcholine. *EEG Clin. Neurophysiol.*, 1: 283-290, 1949
- 6 ANDREWS, H. L. Brain potentials and morphine addiction. *Psychosom. Med.* 3: 399-409, 1941
- 7 ANDREWS, H. L. Cortical effects of demerol (dolantin). *J. Pharmacol. & Exper. Therap.*, 76: 89-94, 1942
- 8 ARVANTZAKI, A. Variations lentes de potentiel associées au fonctionnement rythmique des nerfs non myélinisés, isolés. *J. Physiol. Pathol. gen.*, 34: 1182-1197, 1936
- 9 BAUMSTET, A., BUGNARD, L., GREIER RUEFF, F. A., GREIER RUEFF, CH., AND PLANQUES, J. [The effect of intravenous insulin on the EEG of epileptic] International Electroencephalographic Meeting, London, July, 1947
- 10 BARNES, T. C. AND AMOROSO, M. D. Use of pentothal in electroencephalography of babies. *Fed. Proc.*, 6: 74-75, 1947
- 11 BARNES, T. C., RUTH, H. S., AND HOLTMAN, R. Electroencephalograph of infants under pentothal anesthesia. *Fed. Proc.*, 5: 6, 1946
- 12 BEAN, J. W. Effects of oxygen at increased pressure. *Physiol. Rev.*, 25: 1-147, 1945
- 13 BECHTER, H. K. Chemical constitution and anesthetic potency in relation to cortical potentials. *J. Neurophysiol.*, 3: 347-358, 1940
- 14 BECHTER, H. K. AND McDONOUGH, F. K. Cortical action potentials during anesthesia. *J. Neurophysiol.*, 2: 289-307, 1939
- 15 BECHTER, H. K., McDONOUGH, F. K., AND FORBES, A. Similarity of effects of barbiturate anesthesia and spinal transection. *J. Neurophysiol.*, 2: 81-88, 1939
- 16 BELNAP, W. D., TOMAN, J. E. P., AND GOODMAN, L. S. Tridione-diphenylhydantoin combination therapy in grand mal with slow wave EEG dysrhythmia. *Fed. Proc.*, 6: 305-309, 1947
- 17 BENNETT, A. L. AND CHINBERO, K. G. The effects of several local anesthetics on the resting potential of isolated frog nerve. *J. Pharmacol. & Exper. Therap.*, 88: 72-81, 1946
- 18 BERGER, F. M. Spinal cord depressant drugs. *Pharmacol. Rev.*, 1: 243-278, 1949
- 19 BERGER, F. M. AND BRADLEY, W. Muscle-relaxing action of myanesin. *Lancet* 1: 97, 1947
- 20 BERGER, H. Ueber das Elektroencephalogramm des Menschen I. Mitteilung. *Arch. f. Psychiat.*, 87: 537-570, 1929
- 21 BERGER, H. Ueber das Elektroencephalogramm des Menschen. III. Mitteilung. *Arch. f. Psychiat.*, 94: 16-60, 1931
- 22 BERGER, H. Ueber das Elektroencephalogramm des Menschen. IX. Mitteilung. *Arch. f. Psychiat.*, 102: 538-557, 1934
- 23 BERTRAND, I., DELAT, J., AND GUILLAIN, J. L. Electroencephalogramme dans le myxoedème. *Compt. rend. Soc. de biol.*, 129: 395-398, 1938
- 24 BERTRAND, I., DELAT, J., AND GUILLAIN, J. L. Electroencephalogramme. normal et pathologique Masson et Cie, Paris, 1939
- 25 BIRROF, G. H. Action of nerve depressants on potential. *J. Cell Comp. Physiol.*, 1: 177-194, 1932
- 26 BIRROF, G. H. The interpretation of cortical potentials. *Cold Spring Harbor Symp. Quant. Biol.* 4: 305-319, 1936
- 27 BLIER, J. S. AND REDLICH, F. C. Electroencephalograms in the pitressin hydration test for epilepsy. *Arch. Neurol. & Psychiat.*, 57: 211-219, 1947
- 28 BONNET, V. AND BREMER, F. Action du potassium, du calcium et de l'acétylcholine sur les activités électriques spontanées et provoquées de l'écorce cérébrale. *Compt. rend. Soc. de biol.*, 126: 1271-1275, 1937
- 29 BOROKOWSKI, W. J. AND FORSTER, F. M. Convulsant effects of penicillin on the cerebral cortex. *J. Neuropath. Exper. Neurol.* 6: 201-203, 1947
- 30 BORSTEIN, M. B. Presence and action of acetylcholine in experimental brain trauma. *J. Neurophysiol.*, 9: 349-366, 1946
- 30a. BOYABET, L. L., ROSENBLATT, A. D., POSTEL, S. AND GERARD, R. W. Action of methyl fluoracetate on respiration and potential of nerve. *Am. J. Physiol.*, 157: 291-298, 1949
- 31 BRAKIER, M. A. B. The physiological effects of carbon dioxide on the activity of the central nervous system in man. *Medicine*, 22: 205-221, 1943
- 32 BRAKIER, M. A. B. Studies on the physiology of fight: effect of anoxia on the E.E.G. of psychoneurotic and normal adults. *Arch. Neurol. & Psychiat.*, 56: 725-729, 1946

- 33 BRAZIER M A B Physiological mechanisms underlying the electrical activity of the brain. *J Neurol., Neurosurg., & Psychiat.* 11 118-133, 1948
- 34 BRAZIER M A B AND FINESINGER, J E Action of barbiturates on the cerebral cortex. *Electroencephalographic studies Arch Neurol & Psychiat.* 53 51-59, 1945
- 35 BRAZIER, M A B, FINESINGER, J, AND SCHWAB, R S Characteristics of the normal electroencephalogram. II. The effect of varying blood sugar levels on the occipital cortical potentials in adults during quiet breathing. *J Clin Invest.* 23 313-317, 1944
- 36 BRAZIER M A B, FINESINGER, J E, AND SCHWAB, R S Characteristics of the normal electroencephalogram. III The effects of varying blood sugar levels on the occipital cortical potentials in adults during hyperventilation. *J Clin Invest.* 23 319-323 1944
- 37 BREMER F Action de différentes narcotiques sur les activités électriques spontanées et réflexes du cortex cérébral. *Compt rend Soc. de biol.* 121 861-866, 1936
- 38 BREMER F Action de la strychnine en application locale sur l'activité électrique du cortex cérébral. *Compt. rend Soc. de biol.* 123 90-95, 1936
- 39 BREMER, F Différence d'action de la narcose étherique et du sommeil barbiturique sur les réactions sensorielles acoustiques du cortex cérébral. Signification de cette différence en ce qui concerne le mécanisme du sommeil. *Compt rend Soc. de biol.* 124 848-852 1937
- 40 BREMER, F Effets de la désafférentation complète d'une région de l'écorce cérébrale sur son activité électrique spontanée. *Compt rend Soc de biol.* 127 355-358, 1938
- 41 BREMER, F Parenté des diverses ondes électriques de l'écorce cérébrale. *Compt. rend Soc. de biol.* 128: 544-549, 1938
- 42 BREMER, F 1947 Cited in Walter, W G and Walter, V J (331)
- 43 BREMER F Considérations sur l'origine et la nature des "ondes" cérébrales. *EEG Clin Neurophysiol.* 1 177-193 1949
- 44 BREMER, F and THOMAS, J Action de l'anoxémie, de l'hypercapnie et de l'acapnie sur l'activité électrique du cortex cérébral. *Compt. rend Soc. de biol.* 123. 1256-1261 1936
- 45 BRENNER, C AND MERRITT, H H Effect of certain choline derivatives on electrical activity of cortex. *Arch. Neurol & Psychiat.* 48 382-395 1942
- 46 BRILL, N Q AND SEIDEMANN, H. Electroencephalographic changes during hyperventilation in epileptic and non-epileptic disorders. *Ann. Int. Med.* 16 451-461 1942
- 47 BRINK, F, JR, BRINK D W, AND LARHABEE M G Chemical excitation of nerve. *Ann N Y Acad Sc.* 47 467-485, 1946
- 48 BROOKS, C McC AND ECCLES, J C A study of the effects of anaesthesia and asphyxia on the mono-synaptic pathway through the spinal cord. *J Neurophysiol.* 10 349-360, 1947
- 49 BROOKS, C McC AND ECCLES, J C An electrical hypothesis of central inhibition. *Nature* 159: 760-764, 1947
- 50 BROOKS, V B AND RANNEMIER, R E Enzyme inhibitors on electric activity of frog brain. *Fed. Proc.* 7: 15, 1948.
- 50a. BROOKS, V B., RANNEMIER, R E AND GERARD R W Action of anticholinesterases, drugs and intermediates on respiration and electrical activity of isolated frog brain. *Am J Physiol.* 157 299-316, 1949
- 51 BROWNSTEIN S R. AND PACELLA, B L Convulsions following abrupt withdrawal of barbiturate. *Clinical and electroencephalographic studies. Psychiat. Quart.* 17 112-122, 1943
- 52 CHADWICK, L E AND HILL, D L Inhibition of cholinesterase by diisopropyl fluorophosphate, physostigmine and hexaethyl tetraphosphate in the roach. *J Neurophysiol.* 10 238-246 1947
- 53 CHATFIELD, P O AND DEMPSEY, E W Some effects of prostigmine and acetylcholine on cortical potentials. *Am. J Physiol.* 135 633-640 1942.
- 54 CHENOWETH M B Monofluoroacetic acid and related compounds. *Pharmacol. Rev.* (In press), 1949
- 55 CHENOWETH, M B AND GILMAN, A. Pharmacology of fluoroacetate. *Fed. Proc.* 5 171, 1946
- 56 CHENOWETH M B AND ST JOHN, E F Spike and dome formations produced in the dog's electroencephalogram by fluoroacetic acid. *Proc. Assoc. Res. Nerv. Ment. Dis.* 26 299-307, 1947
- 57 CHENOWETH, M B AND ST JOHN, E F Studies on the pharmacology of fluoroacetate. III Effects on the central nervous systems of dogs and rabbits. *J Pharmacol. & Exper. Therap.* 90 76-82, 1947
- 58 CHURCHILL J A. AND GAMMON, G D The effect of antihistaminic drugs on convulsive seizures. *J Am M A* 141 18-21, 1949
- 59 CHWETZTER, A., GEBLEWICK E, AND LIEBERSON, W Action de la mescaline sur les ondes  $\alpha$  (rythme de Berger chez l'homme. *Compt. rend Soc. de biol.* 124 1295-1299 1937
- 60 CLARK, S L. AND WARD, J W Electroencephalogram of different cortical regions of normal and anesthetized cats. *J Neurophysiol.* 8 99-112 1945
- 61 COHEN, L H THALE, T AND TISSENBAUM, M. J Acetylcholine treatment of schizophrenia. *Arch Neurol & Psychiat.* 51 171-175 1944
- 62 COHN, R. Influence of the harmonic content on the wave forms of the human electroencephalogram. *J Neurophysiol.* 9: 161-184, 1946
- 63 COHN, R. AND GERBER, I Changes in brain potentials during convulsions induced by oxygen under pressure. *J Neurophysiol.* 8. 153-160, 1945.
- 64 COHN, R AND KATZENELBOOM S. Electroencephalographic changes induced by intravenous sodium amytal. *Proc. Soc. Exper Biol Med.* 49: 560-563, 1942
- 65 COHN, R, KOLB, L C, AND MULDER, D W Electroencephalographic changes induced by water intoxication. *J Nerv. Ment. Dis.* 106: 513-526, 1947
- 66 CRESCITELLI, F AND GILMAN, A. Electrical manifestations of the cerebellum and cerebral cortex following DDT administration in cats and monkeys. *Am J Physiol.* 147 127-137, 1946

- 61 CREIGHTILL, F., KOELLE, G. B., AND GILMAN, A. Transmission of impulses in peripheral nerves treated with di-isopropyl fluorophosphate (DFP). *J. Neurophysiol.* 9: 241-252, 1946.
- 62 CRUS, C. H. AND GREENBLATT, M. Absence of alteration in the EEG with stilboesterol and progesterone. *Proc. Soc. Exper. Biol. Med.*, 60 139, 1945.
- 63 CURTIS, H. J. Analysis of cortical potentials mediated by corpus callosum. *J. Neurophysiol.* 3 414-422, 1940.
- 64 CUTLER, K. K. AND JASPER, H. H. Effect of benzedrine sulfate and phenobarbital on behavior problem children with abnormal electroencephalograms. *Arch. Neurol. & Psychiat.* 41 1133-1143, 1939.
- 65 DARROW, C. W. The electroencephalogram and psychophysiological regulation in the brain. *Am. J. Psychiat.* 101 791-793, 1945.
- 66 DARROW, C. W. Psychological and psychophysiological significance of the electroencephalogram. *Psychol. Rev.* 54 157-163, 1947.
- 67 DARROW, C. W., GREEN, J. R., DAVIS, E. W., AND GAROL, H. W. Parasympathetic regulation of high potential in the electroencephalogram. *J. Neurophysiol.* 7 217-228, 1944.
- 68 DARROW, C. W., PATHMAN, J. H., AND KRONENBERG, G. Improvement of the electroencephalogram by atropine. *Fed. Proc.* 4 16, 1945.
- 69 DAVIS, H., DAVIS, P. A., LOOMIS, A. L., HARVEY, E. N., AND HOBART, G. Human brain potentials during the onset of sleep. *J. Neurophysiol.* 1 24-38, 1938.
- 70 DAVIS, H. AND WALLACE, W. McL. Factors affecting the electroencephalographic changes induced by hyperventilation. *Am. J. Physiol.* 133 253-259 1941.
- 71 DAVIS, H. AND WALLACE, W. McL. Factors affecting changes produced in electroencephalogram by standardized hyperventilation. *Arch. Neurol. & Psychiat.* 47 603-625, 1942.
- 72 DAVIS, J. P. Unpublished observations 1949.
- 73 DAVIS, J. P. AND LENNOX, W. G. The effect of trimethylloxazolidine dione and of dimethylethylloxazolidine dione on seizures and on the blood. *Proc. Assoc. Res. Nerv. Ment. Dis.* 26 423-436, 1947.
- 74 DAVIS, P. A. Effect on the electroencephalogram of alterations of blood sugar level. *Am. J. Physiol.* 133 252-260 1941.
- 75 DAVIS, P. A. Effect on the electroencephalogram of changing the blood sugar level. *Arch. Neurol. & Psychiat.* 49 183-184, 1943.
- 76 DAVIS, P. A., DAVIS, H., AND THOMPSON, J. W. Progressive changes in the human electroencephalogram under low oxygen tensions. *Am. J. Physiol.* 123 51-52, 1933.
- 77 DAVIS, P. A., GIBBS, F. A., DAVIS, N., JETTER, W. W., AND THORNBOROUGH, L. S. Effects of alcohol upon electroencephalogram (brain waves). *Quart. J. Stud. on Alcohol*, 1 626-637, 1941.
- 78 DAVIS, P. A. AND SULZACK, W. Changes in the electroencephalogram during metrazol therapy. *Arch. Neurol. & Psychiat.* 43 341-353, 1940.
- 79 DEMPSEY, E. W. AND MORISON, R. S. The production of rhythmically recurrent cortical potentials after localized thalamic stimulation. *Am. J. Physiol.* 135 223-300 1942.
- 80 DEMPSEY, E. W. AND MORISON, R. S. The interaction of certain spontaneous and induced cortical potentials. *Am. J. Physiol.* 135: 301-308 1942.
- 81 DEWITT, A. J., MURPHY, F. J., CORRIGAN, K. E., AND LOBELLE, L. Some observations of the effects of nitrous oxide upon the electroencephalogram in man. *Am. J. Physiol.* 133 281-282, 1941.
- 82 DEWITT, A. J., REMPPEL, B., FORBES, A. AND LAMBERT, E. F. The effects of anesthetics on action potentials in the cerebral cortex of the cat. *Am. J. Physiol.* 116 577-596, 1936.
- 83 DOTY, R. W. AND GERARD, R. W. Separate inhibition of resting and active oxygen consumption of functioning nerves. *Fed. Proc.* 8 35-36 1949.
- 84 DROBOSKI, Z. AND DROBOSKA, J. Étude électroencéphalographique de la localisation pharmacologique des narcotiques. *Arch. internat. de pharmacodyn. et de therap.* 62 265-279 1939.
- 85 DUMER DE BARENNE, J. G. Mode and site of action of strychnine in nervous system. *Physiol. Rev.* 13 325-335 1933.
- 86 DUMER DE BARENNE, J. G. AND McCULLOCH, W. S. Some effects of laminar thermocoagulation upon the local action potentials of the cerebral cortex of the monkey. *Am. J. Physiol.* 114 692-694 1936.
- 87 DUMER DE BARENNE, J. G., McCULLOCH, W. S., AND NIME, L. F. Functional activity and pH of the cerebral cortex. *J. Cell. Comp. Physiol.* 10 277-289 1937.
- 88 ECKLES, J. C. Synaptic potentials of motoneurons. *J. Neurophysiol.* 9 87-120 1946.
- 89 ENGEL, G. L., FERRIS, E. B., AND LOGAN, M. Hyperventilation analysis of clinical symptomatology. *Ann. Int. Med.* 27 633-704, 1947.
- 90 ENGEL, G. L. AND ROMANO, J. Delirium II. Reversibility of the electroencephalogram with experimental procedures. *Arch. Neurol. & Psychiat.* 51 378-392 1944.
- 91 ENGEL, G. L., ROMANO, J. AND FERRIS, E. B. Effect of quinsacrine (atabrine) on the central nervous system: clinical and electroencephalographic studies. *Arch. Neurol. & Psychiat.* 58 337-351 1947.
- 92 ENGEL, G. L., ROMANO, J., FERRIS, E. B., WEBB, J. P. AND STEVENS, C. D. A simple method of determining frequency spectrums in the electroencephalogram. Observations of effects of physiologic variations in dextrose, oxygen posture and acid base balance on the normal electroencephalogram. *Arch. Neurol. & Psychiat.* 51 134-146 1944.
- 93 ENGEL, G. L., ROMANO, J., AND GOLDMAN, L. Delirium IV. Quantitative electroencephalographic study of a case of acute arsenical encephalopathy. *Arch. Neurol. & Psychiat.* 56 657-664 1947.
- 94 ENGEL, G. L. AND ROSENBAUM, M. Delirium III. Electroencephalographic changes associated with acute alcoholic intoxication. *Arch. Neurol. & Psychiat.* 53 44-50 1945.
- 95 ENGEL, G. L., WEBB, J. P., AND FERRIS, E. B. Quantitative electroencephalographic studies of anoxia in humans: comparison with acute alcoholic intoxication and hypoglycemia. *J. Clin. Invest.* 24 691-697 1945.

- 33 BRAZIER, M A B Physiological mechanisms underlying the electrical activity of the brain *J Neurol, N surg. & Psychiat.*, 11 118-133, 1948
- 34 BRAZIER, M A B AND FINESINGER, J E Action of barbiturates on the cerebral cortex *Psych. studies Arch Neurol & Psychiat.*, 53 51-58, 1945
- 35 BRAZIER, M A B, FINESINGER, J, AND SCHWAB, R S Characteristics of the normal electroencephalogram. I The effect of varying blood sugar levels on the occipital cortical potentials in adults during quiet *J Clin Invest.*, 23 313-317, 1944
- 36 BRAZIER, M A B, FINESINGER, J E, AND SCHWAB, R S Characteristics of the normal - *Arch Neurol & Psychiat.*, 53 59-68, 1945
- 37 BREMER, F Action de différentes narcotiques sur les activités électriques spontanées et réflexes du cortex cérébral. *Compt. rend. Soc. de biol.* 121 861-866, 1936
- 38 BREMER, F Action de la strychnine en application locale sur l'activité électrique du cortex cérébral *Compt. rend. Soc. de biol.*, 123 90-95, 1936
- 39 BREMER, F Différence d'action de la narcose étherique et du sommeil barbiturique sur les réactions sensorielles acoustiques du cortex cérébral. Signification de cette différence en ce qui concerne le mécanisme du sommeil. *Compt. rend. Soc. de biol.*, 124 848-852, 1937
- 40 BREMER, F Effets de la désafférentation complète d'une région de l'écorce cérébrale sur son activité électrique spontanée *Compt. rend. Soc. de biol.*, 127 355-358, 1938
- 41 BREMER, F Parenté des diverses ondes électriques de l'écorce cérébrale *Compt. rend. Soc. de biol.*, 128 544-549, 1938
- 42 BREMER, F 1947 Cited in Walter, W G and Walter, V J (331)
- 43 BREMER, F Considérations sur l'origine et la nature des "ondes" cérébrales *EEG Clin Neurophysiol.*, 1 177-193, 1949
- 44 BREMER, F and THOMAS, J Action de l'anoxémie de l'hypercapnie et de l'apnée sur l'activité électrique du cortex cérébral *Compt. rend. Soc. de biol.*, 123 1256-1261, 1936
- 45 BRENNER, C AND MERRITT, H. II Effect of certain choline derivatives on electrical activity of cortex *Arch. Neurol & Psychiat.*, 48 382-395, 1942
- 46 BRILL, N Q AND SEIDEMANN, H. Electroencephalographic changes during hyperventilation in epileptic and non-epileptic disorders *Ann. Int. Med.*, 16: 451-481, 1942
- 47 BRINK, F, JR., BRINK, D W, AND LARRABEE, M G Chemical excitation of nerve *Ann. N. Y. Acad. Sci.*, 47 457-485, 1948
- 48 BROOKS, C McC AND ECCLES, J C A study of the effects of anaesthesia and asphyxia on the mono-synaptic pathway through the spinal cord. *J. Neurophysiol.*, 10 349-380, 1947
- 49 BROOKS, C McC AND ECCLES, J C An electrical hypothesis of central inhibition *Nature* 159: 760-764, 1947
- 50 BROOKS, V B AND RAMSMEIER, R. E Enzyme inhibitors on electro activity of frog brain. *Fed. Proc.*, 7 15, 1948
- 50a BROOKS, V B RAMSMEIER, R. E AND GERARD, R W Action of anticholinesterases, drugs and intermediates on respiration and electrical activity of isolated frog brain *Am. J. Physiol.*, 157 299-316, 1949
- 51 BROWNSTEIN, S R AND PACELLA, B L Convulsions following abrupt withdrawal of barbiturate clinical and electroencephalographic studies *Psychiat. Quart.*, 17: 112-122, 1943
- 52 CHADWICK, L. E AND HILL, D L. Inhibition of cholinesterase by diisopropyl fluorophosphate, physostigmine and hexaethyl tetraphosphate in the roach. *J. Neurophysiol.* 10 238-248, 1947
- 53 CHATFIELD, P O AND DEMPSEY, E W Some effects of prostigmine and acetylcholine on cortical potentials. *Am. J. Physiol.*, 135 633-640, 1942.
- 54 CHENOWETH, M B Monofluoroacetic acid and related compounds *Pharmacol. Rev.*, (In press), 1949
- 55 CHENOWETH, M B AND GILMAN, A. Pharmacology of fluoroacetate. *Fed. Proc.* 5: 171, 1948
- 56 CHENOWETH, M B AND ST JOHN, E F Spike and dome formations produced in the dog's electroencephalogram by fluoroacetic acid *Proc. Assoc. Res. Nerv. Ment. Dis.*, 26 299-307, 1947
- 57 CHENOWETH, M B AND ST JOHN, E F Studies on the pharmacology of fluoroacetate III Effects on the central nervous systems of dogs and rabbits *J. Pharmacol. & Exper. Therap.*, 90 76-82, 1947
- 58 CHURCHILL, J A AND GAMMON, G D The effect of antihistaminic drugs on convulsive seizures *J. Am. M. A.* 141 18-21, 1949
- 59 CHWETZER, A., GEBLEWICK, E., AND LIBERSON, W Action de la mescaline sur les ondes  $\alpha$  (rythme de Berger) chez l'homme. *Compt. rend. Soc. de biol.*, 124 1239-1239, 1937
- 60 CLARK, S L AND WARD, J W Electroencephalogram of different cortical regions of normal and anesthetized cats *J. Neurophysiol.*, 8 99-112, 1945
- 61 COHEN, L. H., THALE, T., AND TISSENBAUM, M. J Acetylcholine treatment of schizophrenia. *Arch. Neurol. & Psychiat.*, 51 171-175, 1944
- 62 COHN, R. Influence of the harmonic content on the wave forms of the human electroencephalogram. *J. Neurophysiol.*, 9: 151-164, 1945
- 63 COHN, R. AND GERSH, I Changes in brain potentials during convulsions induced by oxygen under pressure. *J. Neurophysiol.*, 8. 155-160, 1945
- 64 COHN, R. AND KATZENBROCK, S. Electroencephalographic changes induced by intravenous sodium amytal *Proc. Soc. Exper. Biol. Med.*, 49: 560-563, 1942.
- 65 COHN, R., KOLB, L. C., AND MULDER, D W Electroencephalographic changes induced by water intoxication. *J. Nerv. Ment. Dis.* 106 513-528, 1947
- 66 CRESCITELLI, F AND GILMAN, A. Electrical manifestations of the cerebellum and cerebral cortex following DDT administration in cats and monkeys. *Am. J. Physiol.*, 147 127-137, 1945

132. GIBBS, F. A., GIBBS, E. L., AND LENNOX, W. G. Electroencephalographic response to overventilation and its relation to age. *J. Pediatr.*, 23: 497-505, 1943
133. GIBBS, F. A., GIBBS, E. L., LENNOX, W. G., AND VINE, L. F. The value of carbon dioxide in counteracting the effects of low oxygen. *J. Aviation Med.*, 14: 250-251, 1943
134. GIBBS, F. A. AND MALTBY, G. L. Effect on the electrical activity of the cortex of certain depressant and stimulant drugs—barbiturates, morphine, caffeine, benzedrine and adrenalin. *J. Pharmacol. & Exper. Therap.*, 78: 1-10, 1943
135. GIBBS, F. A., WILLIAMS, D., AND GIBBS, E. L. Modification of the cortical frequency spectrum by changes in  $\text{CO}_2$ , blood sugar and  $\text{O}_2$ . *J. Neurophysiol.*, 3: 49-53, 1940
136. GIRDEN, E. Effect of strychnine muscle paralysis induced with erythroidine upon the electroencephalogram (EEG). *Proc. Soc. Exper. Biol. Med.*, 53: 163-164, 1943
137. GIRDEN, E. The electroencephalogram in curarized mammals. *J. Neurophysiol.*, 11: 159-174, 1943
138. GOLDFARB, W. Effect of the blood sugar on the electroencephalogram. *New York State J. Med.*, 45: 1460-1462, 1945
139. GOLLA, F., GRAHAM, S., AND WALTER, W. G. The electroencephalogram in epilepsy. *J. Ment. Sci.*, 53: 137-155, 1937
140. GOODMAN, L. S. The pharmacodynamic actions of benzimidazole: a preliminary report. *Bull. New Eng. Med. Center*, 5: 97-100, 1943
141. GOODMAN, L. S. AND GHILMAN, A. The Pharmacological Basis of Therapeutics. The Macmillan Co., New York, 1941
142. GOODMAN, L. S. AND MANTEL, C. The anticonvulsive properties of dimethyl N-methyl barbituric acid and 3,5,5-trimethylisoxazolidine-2,4-dione (tridione). *Fed. Proc.*, 4: 119-120, 1945
143. GOODMAN, L. S. AND TOMAN, J. E. P. Experimental indices for comparing the efficacy of compounds with anticonvulsant and antiepileptic properties. *Fed. Proc.*, 4: 120, 1945
144. GOODMAN, L. S. AND TOMAN, J. E. P. Pharmacology of the nervous system. *Prog. Neurol. & Psychiat.*, 1: 149-204, 1946
145. GOODMAN, L. S. AND TOMAN, J. E. P. Pharmacology of the nervous system. *Prog. Neurol. & Psychiat.*, 2: 62-83, 1947
146. GOODMAN, L. S. AND TOMAN, J. E. P. Pharmacology of the nervous system. *Prog. Neurol. & Psychiat.*, 3: 83-110, 1948
147. GOODMAN, L. S. AND TOMAN, J. E. P. Unpublished observations. 1949
148. GOODMAN, L. S., TOMAN, J. E. P., AND SWINTARD, E. A. Anticonvulsant properties of tridione: laboratory and clinical investigations. *Am. J. Med.*, 1: 213-223, 1946
149. GOODMAN, L. S., SWINTARD, E. A., AND TOMAN, J. E. P. Laboratory techniques for the identification and evaluation of potentially antiepileptic drugs. *Proc. Am. Fed. Clin. Res.*, 2: 100-101, 1945
150. GOODMAN, L. S., SWINTARD, E. A., AND TOMAN, J. E. P. Effects of 1 (+) glutamic acid and other agents on experimental seizures. *Arch. Neurol. & Psychiat.*, 56: 20-29, 1945
151. GOODMAN, L. S., SWINTARD, E. A., AND TOMAN, J. E. P. Further studies on the anticonvulsant properties of tridione (3,5,5-trimethylisoxazolidine-2,4-dione). *Fed. Proc.*, 5: 179, 1946
152. GOODMAN, L. S., SWINTARD, E. A., AND TOMAN, J. E. P. Studies on the anticonvulsant properties of diphenylhydantoin. *Fed. Proc.*, 5: 150, 1946
153. GRASS, A. M. AND GIBBS, F. A. A Fourier transform of the electroencephalogram. *J. Neurophysiol.*, 1: 521-526, 1938
154. GREENBLATT, M., LEVIN, S., AND DI CORI, F. The electroencephalogram associated with chronic alcoholism, alcoholic psychosis and alcoholic convulsions. *Arch. Neurol. & Psychiat.*, 52: 290-295, 1944
155. GREENBLATT, M., LEVIN, S., AND SCHLEGLOFF, B. Electroencephalographic findings in bromide intoxication. *Arch. Neurol. & Psychiat.*, 53: 431-435, 1945
156. GREENBLATT, M., MURRAY, J., AND ROOT, H. F. Electroencephalographic studies in diabetes mellitus. *New England J. Med.*, 234: 119-121, 1946
157. GREENBLATT, M. AND ROSE, A. S. Electroencephalographic studies during fever induced by typhoid vaccine and malaria in patients with neurosyphilis. *Am. J. Med. Sc.*, 207: 512-519, 1944
158. GREENELL, R. G. The effects of adrenal cortical extract on the electrical activity of the brain. *Proc. 17 Internat. Physiol. Congress.*, 296-297, 1947
159. GREENELL, R. G. AND McCAWLEY, E. L. The effects of adrenal cortical extract on the electroencephalogram. *Fed. Proc.*, 6: 116-117, 1947
160. GREENELL, R. G. AND McCAWLEY, E. L. Central nervous system resistance. III. The effect of adrenal cortical substances on the central nervous system. *J. Neurosurg.*, 4: 503-518, 1947
161. GRINKER, R. R. AND SEROTA, H. M. Electroencephalographic studies of corticobypothalamic relations in schizophrenia. *Am. J. Psychiat.*, 98: 335-392, 1941
162. GROB, D., HARVEY, A. M., LANOWORTHY, O. R., AND LILIENTHAL, J. L., JR. The administration of diisopropyl fluorophosphate (DFP) to man. III. Effect on the central nervous system with special reference to the electrical activity of the brain. *Bull. Johns Hopkins Hosp.*, 81: 257-266, 1947
163. GROB, D., LILIENTHAL, J. L., JR., AND HARVEY, A. M. The administration of diisopropyl fluorophosphate (DFP) to man. II. Effect on intestinal motility and use in the treatment of abdominal distention. *Bull. Johns Hopkins Hosp.*, 81: 245-256, 1947
164. GROB, D., LILIENTHAL, J. L., JR., HARVEY, A. M., AND JONES, B. F. The administration of diisopropyl fluorophosphate (DFP) to man. I. Effect on plasma and erythrocyte cholinesterase: general systemic effects, use in

- 101 ERICKSON, T C, GILSON, W E, ELVEHJER, C A., AND NEWELL, G W Wheat gluten as a convulsant. *Proc Assoc. Res. Nerv. Ment. Dis.*, 26 164-174, 1947
- 102 EVERETT, G M The effect of d tubocurarine on the central nervous system. *Fed. Proc.*, 6 101, 1947
- 103 EVERETT, G M Pharmacological studies of d tubocurarine and other curare fractions. *J Pharmacol. & Exper. Therap.*, 92: 236-243, 1948
- 104 FAY, T The therapeutic effect of dehydration on epileptic patients. *Arch. Neurol. & Psychiat.*, 23 920-945, 1930
- 105 FEITELBERG, S AND PICK, E P Action of curare on the brain of the frog. *Proc. Soc. Exper. Biol. Med.*, 49: 654-658, 1942
- 106 FELDORNO, W Present views on the mode of action of acetylcholine in the central nervous system. *Physiol. Rev.*, 25 596-642, 1945
- 107 FIAMBERTI A M Accessi a carattere epilettico provocati con l'introduzione sottoccipitale de sostanze vasodilatatrici. *Riv. sper. di freniat.*, 61 834-840, 1937
- 108 FINESINGER, J E AND BRAUER, M A B Vulnerability to drugs of various cortical regions as evidenced by electroencephalograms. *Trans. Am. Neurol. Assoc.*, 70: 151-154, 1944.
- 109 FINESINGER, J E, LINDEMANN, E, BRAUER, M A B, AND CHAPPELLE, E D Effect of anoxia as measured by electroencephalogram and interaction chronogram on psychoneurotic patients. *Am. J. Psychiat.*, 103 738-748, 1947
- 110 FLEISCHL VON MARXOW, E Mittheilung betreffend die Physiologie der Hirnrinde. *Zbl. Physiol.*, 4: 537-540, 1890
- 111 FORSTER, O Hyperventilation-epilepsie. *Deutsche Zeitschr. f. Nervenh.*, 83: 347-356, 1924
- 112 FORBES, A, DERBYSHIRE, A. J, REMPEL, B, AND LAMBERT, E The effects of ether, nembutal and avertin on the potential patterns of the motor cortex. *Am. J. Physiol.*, 113 43-44, 1935
- 113 FORBES, A AND MORISON, B R Cortical response to sensory stimulation under deep barbitalurate narcosis. *J. Neurophysiol.*, 2: 112-123, 1939
- 114 FORSTER, F M Action of acetylcholine on the motor cortex. *Arch. Neurol. & Psychiat.*, 54: 391-394, 1946
- 115 FREEMAN A M, BALES, P D, WILLIS, A, AND HINWICH, H E Experimental production of electrical major convulsive patterns. *Am. J. Physiol.*, 156 117-124, 1949
- 116 FURTER, B, GIBBS, E L, AND GIBBS, F A The electroencephalographic diagnosis and localization of psychomotor epilepsy during sodium pentothal induced sleep. *Trans. Am. Neurol. Assoc.*, June, 1947
- 117 GAILLOUX, L AND LEMAIRE, R. Effect of the ingestion of quinine salts on the electric activity of the cerebral cortex in man. *Compt. rend. Soc. de biol.*, 143 436-437, 1949
- 118 GAMMON G D AND CHURCHILL, J A Effect of myanesein upon the central nervous system. *Am. J. Med. Sc.*, 217: 143-148, 1949
- 118a GASSER, H S AND ERLANGER, J Role of fiber size in establishment of nerve block by pressure or cocaine. *Am. J. Physiol.*, 88: 591-591, 1929
- 119 GELLHORN, E Effects of hypoglycemia and anoxia on the central nervous system. *Arch. Neurol. & Psychiat.*, 40 125-146, 1933
- 120 GELLHORN, E Subcortical centers as pacemakers of cortical activity. *Proc. Soc. Exper. Biol. Med.*, 70 107-103, 1949
- 121 GELLHORN, E AND BALLIN, H M Water intoxication and the electroencephalogram. *Am. J. Physiol.*, 146 559-566, 1946
- 122 GELLHORN, E AND HAILMAN, H The parallelism in changes of sensory function and electroencephalogram in anoxia and the effect of hypercapnia under these conditions. *Psychosom. med.*, 6 23-30, 1944
- 123 GELLHORN, E AND HAYMAN, C Differential action of anoxia, asphyxia and carbon dioxide on normal and convulsive potentials. *J. Neurophysiol.*, 11 261-273, 1948
- 124 GELLHORN, E. AND KESLER, M The effect of hypoglycemia on the electroencephalogram at varying degrees of oxygenation of the blood. *Am. J. Physiol.* 136 1-8 1942
- 125 GELLHORN, E AND YEALINICK, L The significance of carotid sinus reflexes for the effects of anoxia and carbon dioxide on convulsions. *Am. J. Physiol.*, 137 404-403, 1942
- 126 GERARD, R. W Factors influencing brain potentials. *Trans. Am. Neurol. Assoc.*, 62 55-60, 1936
- 127 GERARD, R. W The interaction of neurones. *Ohio J. Sci.*, 41: 160-172, 1941
- 128 GERARD, R. W 1949 Cited in Chanoweth (54)
- 129 GIBBS, E L, GIBBS, F A, LENNOX, W G AND NIME, L F Regulation of cerebral carbon dioxide. *Arch. Neurol. & Psychiat.*, 47: 879-889, 1942
- 130 GIBBS, F A. Electrical activity of the brain. *Ann. Rev. Physiol.*, 7: 427-440, 1945
- 131 GIBBS, F A Electroencephalography. *Am. J. Psychiat.*, 102 527-535 1946
- 132 GIBBS, F A. Electroencephalography. *Am. J. Psychiat.*, 103 519-522, 1947
- 133 GIBBS, F A AND DAVIS, H. Changes in the human electroencephalogram associated with loss of consciousness. *Am. J. Physiol.*, 113: 49-50, 1935
- 134 GIBBS, F A., EVERETT, G M., AND RICHARDS, R. K Phenurone in epilepsy. *Dis. Nerv. System*, 10: 47-49, 1949
- 135 GIBBS, F A. AND GIBBS, E L Atlas of Electroencephalography. Low A. Cummings Co., Cambridge Massachusetts, 1941
- 136 GIBBS, F A., GIBBS, E. L., AND LENNOX, W G Effect on electroencephalogram of certain drugs which influence nervous activity. *Arch. Int. Med.*, 60: 154-165, 1937
- 137 GIBBS, F A., GIBBS, E L., AND LENNOX, W G Cerebral dysrhythmias of epilepsy measures for their control. *Arch. Neurol. & Psychiat.*, 39: 293-314, 1933
- 138 GIBBS, F A., GIBBS, E. L., AND LENNOX, W G Influence of the blood sugar level on the wave and spike formation in petit mal epilepsy. *Arch. Neurol. & Psychiat.*, 41 1111-1116, 1939



206. KNOTT, J. R. AND GOTTLIEB, J. S. Changes in the electroencephalogram following insulin shock therapy, a quantitative study. *Arch. Neurol. & Psychiat.*, 50: 535-537, 1943
207. KNOTT, J. R., GOTTLIEB, J. S., LEET, H. H., AND HADLEY, H. D., JR. Changes in the electroencephalogram following metrazol shock therapy a quantitative study. *Arch. Neurol. & Psychiat.*, 50: 529-534, 1943.
208. KOENIG, H. AND GROOT, R. A. Effects of lowering the blood pH on excitability of the nervous system. *J. Neurophysiol.*, 8: 267-272, 1945
209. KORNHÜLLER, A. E. AND NOELL, W. Über den Einfluss der Kohlensäurespannung auf bioelektrische Hirnrindenphänomene. *Arch. ges. Physiol.*, 247: 660-664, 1944
210. KRITZIANSEN, K. AND COUSTOIS, C. Rhythmic electrical activity from isolated cerebral cortex. *EEG Clin. Neurophysiol.*, 1: 265-272, 1949
211. LARRABEE, M. G., POSTERNAK, J. M., AND BROWN, D. W. Effects of chemical agents on metabolism and function of synapses and fibers in sympathetic ganglia. *Fed. Proc.*, 6: 149-149, 1947
212. LÉLO, A. A. P. AND MORRISON, R. S. Propagation of spreading cortical depression. *J. Neurophysiol.*, 8: 33-45, 1945.
213. LEHMANN, J. E. The effect of changes in pH on the action of mammalian A nerve fibers. *Am. J. Physiol.*, 118: 600-612, 1937
214. LEIMDORFER, A. An electroencephalographic analysis of the action of amideone, morphine and strychnine on the central nervous system. *Arch. Internat. de Pharmacodyn. et de thérap.* 76: 153-162, 1949
215. LEMERE, F. Effects on electroencephalogram of various agents used in treating schizophrenia. *J. Neurophysiol.*, 1: 590-595, 1938
216. LENNOX, M. Effects of sedative drugs on the electroencephalogram. *Am. J. Psychiat.*, 102: 792-804, 1946
217. LENNOX, M. A. AND LENNOX, W. G. Electrical activity of the brain. *Ann. Rev. Physiol.*, 9: 507-524, 1947
- 217a. LENNOX, M. A., RUCH, T. C., AND GUTERMAN, B. The effect of benzedrine and other chemical agents upon the postconvulsive (electric shock) EEG. *Fed. Proc.*, 5: 62, 1946.
218. LENNOX, W. G. AND COBB, S. Epilepsy From the standpoint of physiology and treatment. *Medicine* 7: 103-230, 1923
219. LENNOX, W. G. The effect on epileptic seizures of varying the composition of the respired air. *J. Clin. Invest.* 4: 23-24, 1929
220. LENNOX, W. G. The drug therapy of epilepsy. *J. Am. M. A.*, 114: 1347-1354, 1940
221. LENNOX, W. G. The petit mal epilepsies—Their treatment with tridione. *J. Am. M. A.*, 129: 1069-1074, 1945.
222. LENNOX, W. G. Influence of drugs on the human electroencephalogram. *EEG Clin. Neurophysiol.*, 1: 45-61, 1949
223. LENNOX, W. G. AND GIBBS, E. L. Oxygen saturation of the arterial blood in epilepsy. *Arch. Neurol. & Psychiat.*, 35: 1198-1202, 1936.
224. LENNOX, W. G., GIBBS, F. A., AND GIBBS, E. L. Effect on the electroencephalogram of drugs and conditions which influence seizures. *Arch. Neurol. & Psychiat.* 36: 1236-1250, 1936.
225. LENNOX, W. G., GIBBS, F. A., AND GIBBS, E. L. The relationship in man of cerebral activity to blood flow and to blood constituents. *Proc. Assoc. Res. Nerv. Ment. Dis.*, 13: 277-297, 1938
227. LEVY, N. A., SEROTA, H. M., AND GRIMMER, R. R. Disturbances in brain function following convulsive shock therapy. Electro-encephalographic and clinical studies. *Arch. Neurol. & Psychiat.*, 47: 1009-1029, 1942.
228. LIBERSON, W. T. Electroencephalography. *Am. J. Psychiat.*, 104: 457-461, 1943.
229. LIBERSON, W. T. AND STRAUSS, H. Electroencephalographic studies: slow activity during hyperventilation in relation to age. *Proc. Soc. Exper. Biol. Med.* 43: 674-676, 1941
230. LIBET, B. AND GERARD, R. W. Control of the potential rhythm of the isolated frog brain. *J. Neurophysiol.*, 2: 153-169, 1939
231. LINDSLEY, D. B. AND HENRY, C. E. The effect of drugs on behavior and the electroencephalograms of children with behavior disorders. *Psychosom. Med.* 4: 140-149, 1942.
232. LINDSLEY, D. B. AND RUBENSTEIN, B. B. Relationship between brain potentials and some physiological variables. *Proc. Soc. Exper. Biol. Med.* 35: 558-563, 1937
233. LITTON, B. S. AND GIBBS, F. A. Changes in human electroencephalogram produced by sodium cyanide. *Fed. Proc.*, 6: 154, 1947
234. LITTLE, S. C. Changes in the electroencephalogram following administration of mesantoin (methyl phenyl-ethyl hydantoin). *EEG Clin. Neurophysiol.*, 1: 325-332, 1949
235. LIVINGSTON, S., KAUF, L. AND BRIDGES, E. The use of benzedrine and dexedrine sulfate in the treatment of epilepsy. *J. Pediatr.*, 32: 490-494, 1948.
236. LOGAN, G. B. AND BALDES, E. J. Parallel clinical and electroencephalographic improvement in epilepsy—a study of children treated by the ketogenic diet. *Proc. Staff Meet. Mayo Clin.* 17: 345-352, 1942.
237. LOMBA, A., HARVEY, E. V., AND HOBART, G. Electrical potentials of the human brain. *J. Exper. Psychol.*, 19: 239-249, 1938
238. LORENTE DE NÓ, R. A study of nerve physiology. *Pub. Serv. Rockefeller Inst. Med. Research.*, 131 and 132, 1947
239. LORENTE DE NÓ, R. Action potentials of the motoneurons of the hypoglossus nucleus. *J. Cell. Comp. Physiol.*, 29: 207-288, 1947
240. LUBIN, A. J. AND PRICE, J. C. Effect of alkalosis and acidosis on cortical electrical activity and blood flow. *J. Neurophysiol.*, 5: 281-288, 1942.
241. MADDOCK, S., HAWKINS, J. E. AND HOLMES, E. The inadequacy of substances of the "glucose cycle" for maintenance of normal cortical potentials during hypoglycemia produced by hepatectomy with abdominal evisceration. *Am. J. Physiol.* 125: 551-565, 1939

- study of hepatic function and erythropoiesis, and some properties of plasma cholinesterase. *Bull. Johns Hosp*, 81: 217-244, 1947
- 172 HAMPSON, J L, EESIG, C F, WILLIS, A., AND HIMWICH, H. E Effects of di isopropyl fluorophosphate (DFP) on electroencephalogram and cholinesterase activity *Fed. Proc.*, 8: 66-67, 1949
  - 173 VAN HARREVELD, A The electroencephalogram after prolonged brain asphyxiation. *J Neurophysiol.*, 10: 361-370, 1947
  - 174 HARRIS, M M AND PACKELLA, B L Convulsant shock treatment of patients with mental disease by intravenous injection of acetylcholine. *Arch. Neurol. & Psychiat.*, 50: 304-310, 1943
  - 175 HARVEY, A M, LILIENTHAL, J L, JR, GROB, D, JONES, B F, AND TALBOT, S. A The administration of di isopropyl fluorophosphate (DFP) to man IV The effects on neuromuscular functions in normal subjects and in myasthenia gravis. *Bull. Johns. Hopkins Hosp.*, 81: 267-292, 1947
  - 176 HAYSLIP, G W A review of the effects of various drugs on the electroencephalogram. *J Bowman Gray School Med.*, 6: 77-94, 1948
  - 177 HEINBECKER, P AND BARTLEY, S H Manner of strychnine action on the nervous system. *Am. J Physiol.*, 125: 173-187, 1939
  - 178 HEINBECKER, P AND BARTLEY, S H Action of ether and nambutal on the nervous system. *J Neurophysiol.*, 3: 219-236, 1940
  - 179 HENDLEY, C D, DAVENPORT, H W, AND TOMAN, J E P The effect of acid base changes on experimental convulsive seizures. *Am. J Physiol.*, 153: 530-535, 1948
  - 180 HEPPENSTALL, M E Relation between effects of blood sugar level and hyperventilation on electroencephalogram. *J Neurol., Neurosurg. & Psychiat.*, 7: 112-118, 1944
  - 181 HIMWICH, H. E, DALY, C, FASEKAS, J F, AND HERBLICH, H. C The effects of thyroid medication on brain metabolism of cretins. *Am. J Psychiat.*, 98: 489-493, 1942.
  - 182 HIMWICH, H. E, FROSTIO, J P, FASEKAS, J F, HOAGLAND, H, AND HADIDIAN, Z Clinical electroencephalographic and biochemical changes during insulin hypoglycemia. *Proc. Soc. Exper Biol Med.*, 40: 401-402, 1939
  - 183 HIMWICH, H. E, HADIDIAN, Z, FASEKAS, J F, AND HOAGLAND, H Cerebral metabolism and electrical activity during insulin hypoglycemia in man. *Am. J Physiol.*, 125: 578-585, 1939
  - 184 HOAGLAND, H Pacemakers of human brain waves in normals and in general paretics. *Am. J Physiol.*, 116: 604-616, 1936
  - 185 HOAGLAND, H Temperature characteristics of the "Bergar rhythm" in man. *Science*, 83: 84-86, 1936
  - 186 HOAGLAND, H. Electrical brain waves and temperature. *Science*, 84: 139-140, 1936
  - 187 HOAGLAND, H. Brain metabolism and brain wave frequencies. *Am. J Physiol.*, 123: 103, 1938
  - 188 HOAGLAND, H, CAMERON, D, AND RUBIN, A The electroencephalogram of schizophrenics during insulin treatments. The "delta index" as a clinical measure. *Am. J Psychiat.*, 94: 183-208, 1937
  - 189 HOAGLAND, H, HIMWICH, H. E, CAMERON, D, FASEKAS, J F, AND HADIDIAN, Z Effects of hypoglycemia, and pentobarbital sodium on electrical activity of cerebral cortex and hypothalamus (dogs) *J Neurophysiol.*, 2: 276-288, 1939
  - 190 HOAGLAND, H, RUBIN, M. A., AND CAMERON, D. E Electroencephalogram of schizophrenics during insulin hypoglycemia and recovery. *Am. J Physiol.*, 120: 559-570, 1937
  - 191 HOAGLAND, H, RUBIN, M. A., AND CAMERON, D. E Brain wave frequencies and cellular metabolism. Effects of dinitrophenol. *J Neurophysiol.*, 2: 170-173, 1939
  - 192 HOAGLAND, H, RUBIN, M. A., AND CAMERON, D. E Electrical brain waves in schizophrenics during insulin treatments. *J Psychol.*, 3: 513-519, 1937
  - 193 HODOKIN, A. L. The effect of potassium on the surface membrane of an isolated axon. *J Physiol.*, 106: 319-340, 1947
  - 194 HOFFER, P, HOFF, H. H., AND PLUVINAGE, R. I. L. The effect of anticonvulsant therapy on the electroencephalogram of patients with idiopathic epilepsy. *Trans. Am. Neurol. Assoc.*, 185-189, 1947
  - 195 HOFF, H. E AND BRECKENRIDGE, C. G Nature of apneus and the pontine influence on respiration. *Fed. Proc.*, 8: 76, 1949
  - 196 HOFFMAN, W C 1949 Cited in Bridge, E. M *Epilepsy and Convulsive Disorders in Children*. McGraw Hill Book Co., New York, 1949
  - 197 ISABELL, H., EISENHART, A. J, WIKLER, A., AND FRANK, K. The effects of single doses of  $\delta$ -dimethylamino-4,4-diphenyl-3-pentanone (amidone, methadon or 10820) on human subjects. *J Pharmacol. & Exper. Therap.*, 92: 83-89, 1943
  - 198 JARCHO, L. W Excitability of cortical afferent systems in relation to anesthesia. *Fed. Proc.*, 6: 137, 1947
  - 199 JASPER, H, COME, W, PUDENZ, R., AND BENNETT, T The electroencephalograms of monkeys following the application of microcrystalline sulfonamides to the brain. *Surg. Gynec. & Obst.*, 76: 599-611, 1943
  - 200 JASPER, H. H. AND DROOGLEEVER, FORTUYN, J Experimental studies on the functional anatomy of petit mal epilepsy. *Proc. Assoc. Res. Nerv. Ment. Dis.*, 26: 272-293, 1947
  - 201 JASPER, H. H. AND ERICKSON, T. C Cerebral blood flow and pH in excessive cortical discharge induced by metrazol and electrical stimulation. *J Neurophysiol.*, 4: 333-347, 1941
  - 202 JOHNSON, H. C AND WALKER, A. E Intraventricular penicillin. *J. Am. M. A.*, 127: 217-219, 1945.
  - 203 JOHNSON, H. C, WALKER, A. E., CHASE, T. J., AND KOLLROS, J. J Effects of antibiotic substances on the central nervous system. *Arch. Neurol. & Psychiat.*, 55: 184-197, 1946
  - 204 KAUFMAN, I. C, MARSHALL, C., AND WALKER, A. E Activated electroencephalography. *Arch. Neurol. & Psychiat.*, 53: 533-549, 1947
  - 205 KAUFMAN, I. C, MARSHALL, C., AND WALKER, A. E. Metrazol activated electroencephalography. *Proc. Assoc. Res. Nerv. Ment. Dis.*, 26: 476-486, 1947

- 277 PRICE, J. C., WATTS, H., AND PUTNAM, T. J. dl glutamic acid hydrochloride in treatment of petit mal and psychomotor seizures. *J. Am. M. A.*, 122, 1153-1156, 1943
- 278 QUASTEL, J. H. Respiration in the central nervous system. *Physiol. Rev.*, 19, 135-183, 1939
- 279 REXENAW, B., FORBES, A., AND MORISON, B. R. Activity of isocortex and hippocampus. Electrical studies with micro-electrodes. *J. Neurophysiol.*, 3, 74-103, 1940
- 280 RHEINBERGER, M. B. AND JASPER, H. H. The electrical activity of the cerebral cortex in the unanesthetized cat. *Am. J. Physiol.*, 119, 156-198, 1937
- 281 ROEDER, R. D., KENNEDY, N. K., AND SAMSON, E. A. Synaptic conduction to giant fibers of the cockroach and action of anticholinesterases. *J. Neurophysiol.*, 10, 1-10, 1947
- 282 ROBINSON, L. J. Electroencephalographic study during epileptic seizures related to hyperventilation. *Dis. Nerv. System*, 5, 57-90, 1944
- 283 ROBINSON, L. J. Results of repetition of electroencephalography in adult epileptics. *J. Nerv. Ment. Dis.*, 104, 458-458, 1946
- 284 ROMANO, J. AND ENGEL, G. L. Delirium. I. Electroencephalographic data. *Arch. Neurol. & Psychiat.*, 51, 352-377, 1944
- 285 ROSENBLUTH, A. AND CANYON, W. B. Cortical responses to electrical stimulation. *Am. J. Physiol.*, 135, 690-711, 1942
- 286 ROSS, D. A. AND SCHWAB, R. S. The cortical alpha rhythm in thyroid disorders. *Endocrinol.*, 25, 75-79, 1939
- 287 ROSS, A. Cited in Feldberg, W., (104)
- 288 RUBIN, M. A. AND COHEN, L. H. Electroencephalogram in bromide intoxication. *Arch. Neurol. & Psychiat.*, 40, 922-927, 1933
- 289 RUBIN, M. A., COHEN, L. H. AND HOAGLAND, H. The effect of artificially raised metabolic rate on the electroencephalogram of schizophrenic patients. *Endocrinol.*, 21, 336-340, 1937
- 290 RUBIN, M. A. AND FREEMAN, H. The influence of cyanide on brain potentials in man. *J. Neurophysiol.*, 1, 527-532, 1938
- 291 RUBIN, M. A. AND FREEMAN, H. Brain potential changes in man during cyclopropane anesthesia. *J. Neurophysiol.*, 3, 33-42, 1940
- 292 RUBIN, M. A. AND FREEMAN, H. Brain potential changes and skin temperature during cyclopropane anesthesia. *Anesth. & Analg.*, 20, 45-49, 1941
- 293 RUBIN, M. A., MALAMUD, W., AND HOFF, J. M. The electroencephalogram and psychopathological manifestations in schizophrenia as influenced by drugs. *Psychosom. Med.*, 4, 355-361, 1942
- 294 RUBIN, M. A. AND TURNER, E. Blood sugar level and influence of hyperventilation on slow activity in the electroencephalogram. *Proc. Soc. Exper. Biol. Med.*, 50, 270-272, 1942
- 295 SCHLESINGER, E. B., DEWE, A. L., AND WOOD, B. Clinical studies in the use of meprobamate. *Am. J. Med.*, 4, 365-372, 1948
- 296 SCHWITZER, A., STEDMAN, E., AND WRIGHT, S. Central action of anticholinesterases. *J. Physiol.*, 96, 302-336, 1939
- 297 SELYE, H. The antagonism between anesthetic steroid hormones and pentamethylenetetrazol. *J. Lab. Clin. Med.*, 27, 1031-1033, 1942
- 298 SILVER, M. L., JOHNSON, R. E., KARR, R. M., KLEIN, J. R., MONAHAN, E. P., AND ZEVIN, S. S. White bread and epilepsy in animals. *J. Am. M. A.*, 135, 757-760, 1947
- 299 SMOGS, D. J. The use of tridione in the treatment of convulsive disorders. *New York State J. Med.*, 47, 1363-1366, 1947
- 300 SÖSTRAND, T. Potential changes in the cerebral cortex of the rabbit arising from cellular activity and the transmission of impulses in the white matter. *J. Physiol.*, 90, 41-43P, 1937
- 301 SMITH, S. M., BROWN, H. O., TOMAN, J. E. P., AND GOODMAN, L. S. The lack of cerebral effects of di-tubocurarine. *Anesthesiol.*, 8, 1-14, 1947
- 302 SPIEGEL, E. AND SPIEGEL-ADOLF, M. Physicochemical mechanisms in convulsive reactivity. *Proc. Soc. Exper. Biol. Med.*, 34, 799-800, 1936
- 303 STADIE, W. C., RIGGS, B. C., AND HAUGAARD, N. Oxygen poisoning. *Am. J. M. Sc.*, 207, 84-114, 1944
- 304 STELLA, G. The dependence of the activity of the "apneustic centre" on the carbon dioxide of the arterial blood. *J. Physiol.*, 93, 263-275, 1935
- 305 STEPHEN, C. R. AND CHANDY, J. Clinical and experimental studies with meprobamate. *Canad. M. A. J.*, 47, 463-468, 1947
- 306 STRAUSS, H. AND RAHM, W. E. Reactions of the electroencephalogram to meprobamate injections. *Proc. Soc. Exper. Biol. Med.*, 40, 1-2, 1939
- 307 STRAUSS, H. AND WECHSLER, I. S. Clinical and electroencephalographic studies of changes in cerebral function associated with variations in the blood sugar. *Am. J. Psychiat.*, 102, 34-39, 1946
- 308 SUCKLEY, H. M., LIEBENOW, R. R. AND ORTE, O. S. The convulsive effects of streptomycin topically applied to the cerebral cortex. *J. Neurosurg.*, 4, 370-379, 1947
- 309 SUGAR, O. AND GERARD, R. W. Anoxia and brain potentials. *J. Neurophysiol.*, 1, 553-572, 1938
- 310 SWANK, R. L. Synchronization of spontaneous electrical activity of cerebrum by barbiturate narcosis. *J. Neurophysiol.*, 12, 161-172, 1949
- 311 SWANK, R. L. AND FOLTY, J. M. Respiratory, electroencephalographic and blood gas changes in progressive barbiturate narcosis in dogs. *J. Pharmacol. & Exper. Therap.*, 92, 331-396, 1948
- 312 SWANK, R. L. AND WATSON, C. W. Effects of barbiturates and anesthesia on spontaneous electrical activity of dog brain. *J. Neurophysiol.*, 12, 137-160, 1949

- 241a. MALING, H M AND ACHESON, G H. Righting and other postural activity in low-decerebrate and in spinal cats after d amphetamine. *J Neurophysiol*, 9 379-385, 1945.
242. MARRUFO, O AND HELFAND, M. Analysis of one hundred cases of epilepsy *J Nerv Ment. Dis.*, 104. 465-473, 1946
243. MARSHALL, W H. Observations on subcortical somatic sensory mechanisms of cats under nembutal anesthesia. *J Neurophysiol.*, 4: 25-43, 1941
244. MARSHALL, W H, WOOLSEY, C N, AND BARD, P. Observations on cortical somatic sensory mechanisms of cat and monkey *J Neurophysiol.*, 4 1-24, 1941
245. McCULLOCH, W S. Modes of functional organization of the cerebral cortex. *Fed. Proc.*, 6: 443-451, 1947
246. McCULLOCH, W S. Cortico-cortical connections. Chap. VIII in *The Precentral Motor Cortex*, edited by Paul C Bucy 2nd Edition. University of Illinois Press, Urbana, Illinois, 1949
247. MCINTYRE, A. R. Curare, its History, Nature and Clinical Use. University of Chicago Press, Chicago Illinois, 1947
248. MCINTYRE, A. R. Some physiological effects of curare and their application to clinical medicine. *Phynol. Rev.*, 27 464-477, 1947
249. MCINTYRE, A. R., DUNN, A. L., AND TULLAR, P. E. The effect of d tubocurarine on the electrical activity of dogs' brain. *Fed. Proc.*, 5 67, 1946
250. McQUARRIE, I. AND PEILER, D. B. The effects of sustained pituitary antidiuretics and forced water drinking on epileptic children. A diagnostic and etiologic study. *J Clin. Invest.*, 10: 915-940, 1931
251. McQUARRIE, I. Observations on the therapeutic effect of desoxycorticosterone acetate in epilepsy. *Am J Du Child.*, 72 472, 1946
252. MELLANBY, E. Diet and canine hysteria. experimental production by treated flour. *Brit. M. J.*, 2: 835-837, 1946.
253. MILLER, F R, STAVRAKY, G W, AND WOONTON, G A. Effects of eserine acetylcholine and atropine on the electroencephalogram. *Am J Physiol.*, 123 147, 1938
254. MILLER, F R., STAVRAKY, G W, AND WOONTON, G A. Effects of eserine, acetylcholine and atropine on the electrocorticogram. *J Neurophysiol.*, 3: 131-133, 1940
255. MORISON, R. S. AND BASSETT, D. L. Electrical activity of the thalamus and basal ganglia in decorticate cats. *J Neurophysiol.*, 8 309-314, 1945
256. MORISON, R. S., FINLEY, K. H., AND LOTHROP, G. N. Spontaneous electrical activity of the thalamus and other forebrain structures. *J Neurophysiol.*, 6: 243-254, 1943
257. MORUZZI, G. Cited by Feldberg, W, (106)
258. MORUZZI, G. *L'Epilessia Sperimentale*. Nicola Zanichelli (Editor) Monograph Bologna, 1946
259. MORUZZI, G., BROOKHART, J. M., AND SNIDER, R. S. Activity of single units in the cerebellar cortex. *Fed. Proc.*, 8: 113 1949
260. NEWELL, G W, ERICKSON, T C, GILSON, W E, AND ELVEHJEM, C. A. The effect of wheat gluten diet on the electroencephalograms of dogs. *Proc. Soc. Exper. Biol. Med.*, 65: 115-116, 1947
261. NEWELL, G W, ERICKSON, T C, GILSON, W E, GREGORY, S N, AND ELVEHJEM, C. A. Role of "agenized" flour in the production of running fits. *J Am. M. A.*, 135 760-763, 1947
262. NICKERSON, M. The pharmacology of adrenergic blockade. *J Pharmacol. & Exper. Therap.*, Part II, 95 27-101, 1949
263. ORRADOR, S. Effect of hypothalamic lesions on electrical activity of cerebral cortex. *J Neurophysiol.* 6 81-84, 1943
264. O'LEARY, J. L. The role of architectonics in deciphering the electrical activity of the cortex. Chap. III in *The Precentral Motor Cortex*, edited by Paul C Bucy 2nd Edition. University of Illinois Press, Urbana, Illinois, 1949
265. PERLSTEIN, M. A. Tridione: a new experimental drug for treatment of convulsive and related disorders. II. Clinical investigations. *Arch. Neurol. & Psychiat.*, 55: 164-165, 1946
266. PERLSTEIN, M. A. Tridione therapy—special aspects of its clinical use. *Am J Psychiat.*, 104: 247-253, 1947
267. PERLSTEIN, M. A. Personal communication 1949
268. PERLSTEIN, M. A. AND ANDELMAN, M. B. Tridione: its use in convulsive and related disorders. *J. Pediat.*, 29 20-40, 1946
269. PETERSON, E. W, BORNSTEIN, M. B., AND JAFFE, H. H. Effect of morphine sulfate on persons exposed to simulated altitude. *War Med.* 7 23-28, 1945
270. PFEEFFER, C C AND GERSH, I. The prevention of the convulsions of oxygen poisoning by means of drug. Research Project X-192 Report No. 2, Naval Medical Research Institute, Oct. 30 1944
271. PICK, E. P AND HUNTER, J. The action of atabrine on the electrocortico potentials. *J Pharmacol. & Exper. Therap.*, 80: 354-361, 1944
272. PICK, E. P AND MILLER, M. M. Influence of depletion of diffusible electrolytes upon electrical activity of the brain. *J Neurophysiol.*, 8 47-54, 1945
273. PICK, E. P AND UNNA, K. Effect of curare and curare-like substances on central nervous system. *J Pharmacol. & Exper. Therap.*, 83: 59-70, 1945
274. PILCHER, C, ANGELUCCI, R., AND MEACHAM, W. F. Convulsions produced by the intracranial implantation of sulfathiazole. *J Am. M. A.*, 119 927, 1942.
275. PITTS, R. F., MAGOUN, H. W., AND RANSON, S. W. The origin of respiratory rhythmicity. *Am. J Physiol.*, 127: 654-670, 1939
276. PITTS, W AND McCULLOCH, W S. How we know universals, the perception of auditory, and visual forms. *Bull. Math. Biophys.* 9 127-147, 1947

## INDEX

- Adrenaline, Metabolism of, 1
- Adrenergic blockade, 27
- Anticholinesterase drugs, 166
- Bacq, Z M Metabolism of adrenaline, 1
- Berger, F M Spinal cord depressant drugs, 243
- Brain, electrical activity of, Effects of drugs on, 425
- Cardiac glycosides, Metabolic action of, 311
- Chenoweth, Maynard B Monofluoroacetic acid and related compounds, 383
- Davis, Jean P, and Toman, James E P Effects of drugs on electrical activity of brain, 425
- Dihydroxy phenyl alkylamines and epinephrine, Pharmacological activity of, 279
- Drugs and plasma proteins, Interactions of, 102
- Electrical activity of brain, Effects of drugs on, 425
- Epinephrine and related dihydroxyphenylalkylamines, Pharmacological activity of, 279
- Gilman, Alfred, and Koelle, George B Anticholinesterase drugs, 166
- Goldstein, Avram Interactions of drugs and plasma proteins, 102
- Heart, failing, Energy metabolism of, and metabolic action of cardiac glycosides, 311
- Koelle, George B, and Gilman, Alfred Anticholinesterase drugs, 166
- Lands, A M Pharmacological activity of epinephrine and related dihydroxyphenylalkylamines, 279
- Maynert, E W, and van Dyke, H B Metabolism of barbiturates, 217
- Metabolism of adrenaline, 1
- Monofluoroacetic acid and related compounds, 383
- Nickerson, Mark Pharmacology of adrenergic blockade, 27
- Plasma proteins and drugs, Interactions of, 102
- Proteins, plasma, and drugs, Interactions of, 102
- Salicylates, Certain aspects of pharmacology of, 353
- Smith, Paul K Certain aspects of pharmacology of salicylates, 353
- Spinal cord depressant drugs, 243
- Toman, James E P, and Davis, Jean P Effects of drugs on electrical activity of brain, 425
- van Dyke, H B, and Maynert, E W Metabolism of barbiturates, 217
- Wollenberger, Albert Energy metabolism of failing heart and metabolic action of cardiac glycosides, 311

## ERRATUM

An error in the dose figure for *L*-arterenol was inadvertently made in the review article by A. M. Lands, "The Pharmacological Activity of Epinephrine and Related Dihydroxyphenylalkylamines," in the September, 1949 issue of *Pharmacological Reviews*, Vol. 1, No. 2

On page 284, last line, the dose by intravenous injection is given as 10-20 mcg/kg/min. The dose should be 10-20 mcg/min.

- 313 SWINTARN, E A Personal communication 1949
- 314 SWINTARN, E A, TOMAN, J E P, AND GOODMAN, L S The effect of cellular hydration on experimental electroshock convulsions *J Neurophysiol*, 9: 47-54, 1946
- 315 TOMAN, J E P Cortical responses to cortical stimulation in relation to the spontaneous EEG of the rabbit. *Fed Proc*, 4 72, 1945
- 316 TOMAN, J E P The neuropharmacology of antileptics *EEG Clin Neurophysiol*, 1: 33-44, 1949
- 317 TOMAN, J E P Unpublished observations. 1949
- 318 TOMAN, J E P Neuropharmacology of peripheral nerve. To be published, 1950
- 319 TOMAN, J E P, BELNAP, W D, AND HENRIK, L M Unpublished observations. 1949
- 320 TOMAN, J E P AND GOODMAN, L S Conditions modifying convulsions in animals. *Proc. Assoc. Res. Nerv Ment. Dis*, 26: 141-163, 1947
- 321 TOMAN, J E P AND GOODMAN, L S Anticonvulsants. *Physiol. Rev*, 28: 409-432, 1948
- 322 TOMAN, J E P, GOODMAN, L S, AND SWINTARN, E A Observations on the central excitatory effects of metrazol *Fed Proc*, 5: 208, 1946
- 323 TOMAN, J E P, WOONNURY, J W, AND WOONNURY, L A Mechanism of nerve conduction block produced by anticholinesterases *J Neurophysiol*, 10: 429-441, 1947
- 324 WAGNER, R W On the role of glutamic acid in epilepsy *Fed Proc*, 6 220, 1947
- 325 WAGNER, J R AND ELVEHJEM, C A A study of canine hysteria produced by feeding certain baked dog foods and wheat gluten flour *J Nutrition*, 28 431-441, 1944
- 326 WALKER, A E. AND JOHNSON, H C Convulsive factor in commercial penicillin. *Arch Surg*, 50: 69-73, 1945.
- 327 WALKER, A E. AND JOHNSON, H C Penicillin in Neurology Charles C Thomas, Springfield, Illinois, 1945
- 328 WALKER, A E AND JOHNSON, H C The effect of antibiotic substances on the central nervous system. *Prog Neurol & Psychiat*, 1: 205-210, 1946
- 329 WALKER, A E, JOHNSON, H C, AND KOLLROS, J J Penicillin convulsions The convulsive effects of penicillin applied to the cerebral cortex of man and monkeys. *Surg, Gynec. & Obst.*, 81 692-701, 1945
- 330 WALTER, W G An automatic low frequency analyser *Electr Eng*, 16: 9-13, 1943
- 331 WALTER, W G AND WALTER, V J The electrical activity of the brain *Ann. Rev Physiol*, 11 199-230, 1949
- 332 WARR, A. A., JR. Convulsive activity induced by fluoroacetate. *J Neurophysiol*, 10: 105-111, 1947
- 333 WARD, A. A., JR. AND WHEATLEY, M D Sodium cyanide Sequence of changes of activity induced at various levels of the central nervous system *J Neuropath. Exper Neurol.*, 6: 292-294, 1947
- 334 WATT, A C AND ALEXANDER, G L Epilepsy following application of sulphathiazole near the brain. *Lancet*, 1 493-496, 1942
- 335 WESCOM, W C, GREEN, R. E, McNAMARA, B P, AND KROP, S. The influence of atropine and scopolamine on the central effects of DFP *J Pharmacol. & Exper Therap*, 92 63-72, 1945
- 336 WESTFALL, B A Effects of phenobarbital on oxygen consumption of brain slices *J Pharmacol.*, 96 193-197, 1949
- 337 WHITTAKER, D The role of acetylcholine in synaptic transmission a critical review *J Neurol. Neurosurg. & Psychiat.*, 11: 134-140, 1948
- 338 WIKLER, A Studies on the action of morphine on the central nervous system of the cat. *J Pharmacol. & Exper Therap*, 80: 176-187, 1944
- 339 WIKLER, A. Hindlimb reflexes in chronic spinal dogs during a cycle of morphine addiction *Fed Proc*, 4:141, 1945
- 340 WIKLER, A Effects of morphine on somatic motor components of "sham rage" in chronic decorticate cats and dogs. *Fed. Proc.*, 4 141, 1945
- 341 WIKLER, A Effects of morphine, nembutal, ether, and eserine on two-neuron and multi-neuron reflexes in the cat. *Proc. Soc. Exper Biol Med*, 58: 193-196, 1945.
- 342 WIKLER, A Effects of picrotoxin hydration on electroencephalogram paroxysmal slow activity in non-epileptic patients with previous drug addiction. *Arch. Neurol. & Psychiat.*, 57: 78-83, 1947
- 343 WILLIAMS, D The effect of choline-like substances on the cerebral electrical discharges in epilepsy *J Neurol Neurosurg. & Psychiat.*, 4 32-47, 1941
- 344 WILLIAMS, D Effect of choline-like substances upon epilepsy *J Physiol*, 99 8P, 1941
- 345 WILLIAMS, D AND RUSSELL, W R. Action of eserine and prostigmine on epileptic cerebral discharges. *Lancet*, 1 476-479, 1941
- 346 WOONNURY, L A Early components of contralaterally evoked cortical potentials in the intact unanesthetized rat. *Fed. Proc.*, 8: 172, 1949
- 347 WRIGHT, E B The effects of asphyxiation and narcotics on peripheral nerve polarization and conduction. *Am J Physiol.*, 143 174-184, 1947
- 348 ZISKIND, E, SJAARDEMA, H, AND BERCKEL, N A Minimal electroencephalographic response to metrazol as method for measuring the convulsive threshold for use in human beings. *Science*, 104: 462-463, 1946
- 349 ZISKIND, E AND BERCKEL, N A. Preconvulsive paroxysmal electroencephalographic changes after metrazol injection. *Proc. Assoc. Res. Nerv Ment. Dis*, 26 487-501, 1947

